THE DOPAMINERGIC NETWORK AND GENETIC SUSCEPTIBILITY TO SCHIZOPHRENIA

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Background: Schizophrenia is a disabling illness with unknown pathogenesis. Estimates of heritability suggest a substantial genetic contribution; however genetic studies to date have been equivocal. Uncovering liability loci may therefore require analyses of functionally related genes. Rooted in this assumption, this dissertation describes a series of studies investigating a genetic epidemiological foundation for the commonly cited hypothesis suggesting dopaminergic dysfunction in schizophrenia pathogenesis, i.e. the 'dopamine hypothesis'.

Studies: The initial study investigated *DRD3* and identified novel associations across the gene. The second study considered a larger network of dopaminergic genes in two independent Caucasian samples, detecting replicated associations and epistatic interactions. The study proposed a risk model for schizophrenia centered on the dopamine transporter. Study #3 investigated a dopamine precursor, phenylalanine hydroxylase, in four independent samples, identifying a single SNP (rs1522305) that was significantly replicated in two samples. Study #4 was motivated the hypothesis of a shared genetic etiology for schizophrenia and bipolar disorder. This study comprehensively evaluated the dopaminergic network, selecting 431 'tag' SNPs from 40 genes among large schizophrenia and bipolar cohorts contrasted with adult controls. Across all genes 60% of nominally significant schizophrenia risk factors were also associated with

bipolar disorder. The results supported *DRD3* variations as risk factors for both disorders, confirmed several previously reported associations, and proposed new targets for future research.

Conclusion: These results suggest dopaminergic gene variations could play an etiological role in the pathogenesis of schizophrenia and possibly bipolar 1 disorder. Additional replicate studies are warranted

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Public Health Significance:

Schizophrenia (SZ) is devastating. When the *Global Burden of Disease* study calculated disability adjusted life years, weighted for the severity of disability, they determined active psychosis seen in schizophrenia produces disability equal to quadriplegia. Schizophrenia has been estimated to be among the top ten causes of disability worldwide. As schizophrenia is common (roughly 1% point prevalence worldwide), the economic burden to society is substantial. Pathogenesis is unknown and treatment is palliative. Therefore understanding the genetic etiology could facilitate development of promising therapeutics.

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PREFACE

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This work is dedicated to my parents, who have been constant pillars of support, to my wife, who always believes in me more than is justified, and to my son, Tyler.

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1.0 INTRODUCTION

These data describe an ongoing series of studies aimed at evaluating the genetic epidemiological evidence for or against the long held hypothesis that alterations in dopamine neurotransmission represent a pathogenic mechanism for schizophrenia. The hypotheses and analyses presented here are not novel. Biological studies have long suggested dopamine dysfunction in schizophrenia etiology, and dopamine receptors are primary targets of antipsychotics. Genetic studies have targeted dopamine gene polymorphisms for the better part of the last two decades. However, a review of existing data suggests a substantial gap in knowledge between relatively small single variant association studies and large scale genome-wide association studies. The purpose of these studies is therefore to comprehensively reconsider an existing hypothesis that remains cursorily evaluated.

1.1 PUBLIC HEALTH SIGNIFICANCE

Schizophrenia (SZ) is devastating. When the *Global Burden of Disease* study calculated disability adjusted life years, weighted for the severity of disability, they determined active psychosis seen in schizophrenia produces disability equal to quadriplegia (C. J. L. Murray & Lopez, 1996). One study estimated schizophrenia was among the top ten causes of disability worldwide (Lopez et al., 1998). As schizophrenia is common (1% point prevalence), the

economic burden to society is substantial. In 2002, the US healthcare system spent over $62 billion on schizophrenia patients (Wu et al., 2005) who occupy 30% of all U.S. psychiatric hospital beds. Pathogenesis is unknown and treatment is palliative. Therefore understanding the genetic etiology could facilitate development of promising therapeutics.

The comprehensive nature of individual gene evaluations conducted herein will also impact future studies in other diseases. Dopaminergic gene variants have been proposed as risk factors in diseases such as Alzheimer's, Parkinson's, and ADHD, to name a few. The extensive gene mapping, polymorphism discovery, and linkage disequilibrium analyses in these studies provide an established set of genetic variants representative of all currently available common polymorphisms within the dopaminergic pathway. Future studies can utilize this resource as a source of uniform variations required to test a common variant hypothesis for any spectrum of phenotypic traits of interest.

1.2 SCHIZOPHRENIA

1.2.1 History

Schizophrenia is a psychiatric disorder characterized by psychotic phenomena. Without formal definition, observations of 'diseases of the mind' stretch as far back as Hippocrates (460 – 370 B.C.) (Palha & Esteves, 1997). Clinical pathologies such as mania and melancholia were recognized as forms of madness (I. Gottesman, 1991). The term dementia praecox was first used in 1857 by Benedict Morel (I. Gottesman, 1991). Karl Kahlbaum (1828 – 1899) studied the course of dementia praecox and documented clinical psychoses during all stages of the illness,

being one of the first to suggest the evolution of psychosis was a measurable symptom. Based on the works of these and others, in 1896 Emil Kraeplin expanded Morel's model of dementia praecox and identified a new nosologic systemization of mental illness. Although Kraeplin's broad categories of dementia praecox and manic depressive insanity have since been redefined (Kraepelin, 1919), the essential features of his concept of dementia praecox are present today in the DSM-IV diagnosis of schizophrenia (A.P.A., 1994) (A. Jablensky, 1997) (see (Palha & Esteves, 1997) for review).

1.2.2 Clinical presentation

Schizophrenia is a highly heterogeneous disorder in which the onset is commonly in late adolescence. The pathogenesis of the disorder is unknown and there is no available biomarker or diagnostic test, so diagnoses remain reliant on patient interviews and self-report. Prior to 1980, diagnostic reliability varied widely, particularly across cultures (Cooper & Sartorius, 1977) (Kuriansky, Deming, & Gurland, 1974). The reliability has dramatically improved since publication of the third edition of the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition* (DSM-III) (A.P.A., 1980). Further improvements in the most recent version of the DSM (DSM-IV) have established specific diagnostic criteria (A.P.A., 1994). The first criterion state that two or more of the following symptoms must be present for a significant portion of time during a one-month period (or less if successfully treated) (Criterion A): delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and can include negative symptoms such as flattened affect, alogia (poverty of speech), or avolition (poverty of desire or motivation). Criterion B (*social / occupational dysfunction)* states that symptoms must lead to a disturbance in one or more major areas of functioning (e.g. work, interpersonal

relationships, or self care in adults) for a significant portion of time since their onset. Criterion C (*duration*) requires continuous signs of disturbance that persist for at least six months, including one month of 'active-phase' symptoms (meeting Criterion A). Criterion D and E exclude other disorders such as schizoaffective and mood disorders with psychotic features, and require the disturbance is not a result of direct physiological effects of substance abuse (e.g. drug abuse or medication) or a general medical condition. Criterion F requires that if Autistic Disorder or another Pervasive Developmental Disorder were previously diagnosed, schizophrenia can only be made as an additional diagnosis if prominent delusions or hallucinations are also present for at least a month. There are subtypes of schizophrenia not discussed in detail here, including paranoid, catatonic, disorganized, undifferentiated, and residual type. Of note for the studies presented here, individuals diagnosed with schizoaffective disorder meet Criterion A for schizophrenia, but also have a period during which diagnostic criteria of Major Depressive Episode, Manic Episode, or Mixed Episode is met concurrently.

Other symptoms are often seen that lead to chronic impairment, including deficits in neurocognitive domains such as executive function, attention, and memory (Carpenter, 1994; Peuskens, Demily, & Thibaut, 2005; Sharma & Antonova, 2003). The course and outcome of schizophrenia are as varied as its symptoms. A series of studies published between 1972 and 1985 in European and United States Caucasian populations found differing outcomes between patients, irrespective of diagnostic criteria used (see (Huber, 1997) for review). Taken together, these studies suggest that full psychopathological remission is seen in about 25% of patients and roughly 50% of patients display social remission. These studies also concluded that course of illness could not be reliably predicted at age of onset (Huber, 1997). Based on the conclusions of a series of cross-national World Health Organization (WHO) studies, it has been accepted that

patients in developing countries have a better course and outcome (A. Jablensky et al., 1992; W.H.O., 1979) (G. Harrison et al., 2001). However, a recent analysis of 23 longitudinal studies of schizophrenia outcomes suggests the data is more complex than originally interpreted and those initial findings should be re-examined (A. Cohen, Patel, Thara, & Gureje, 2008). It has also been well accepted that women experience a better outcome than men (R. Z. Cohen, Gotowiec, & Seeman, 2000). The study by Cohen and colleagues found this to be the case in some, but not all countries (A. Cohen et al., 2008), further supporting a complex pattern of course and outcome of schizophrenia based on a myriad of factors.

1.2.3 Epidemiology of schizophrenia

The etiology of schizophrenia is poorly understood, but clearly complex and likely to involve major genetic and environmental contributions. Schizophrenia occurs in all populations studied to date. The lifetime prevalence is often referenced as 1%, and incidence rates have varied from 0.16 – 0.42 across populations (A. Jablensky, 2000), however there have been significant differences in estimates between studies. Although the incidence is often quoted as stable worldwide, one systematic review suggested significant variation in incidence rates around the world (McGrath, 2006). Another review suggested the median lifetime prevalence was only about 0.4% among studies (Saha, Chant, Welham, & McGrath, 2005). A recent population based survey of more than 8,000 individuals from Finland suggested significant differences between psychotic disorders, age groups, and gender within the population studied (Perala et al., 2007), lending further credence to the need for diagnostic reliability highlighted in section 1.2.2 above. Specifically, the authors found a lifetime prevalence of schizophrenia that was slightly

lower than 1% (0.83%); however the lifetime prevalence of all psychotic disorders was roughly 3%. A summary of these findings are detailed in Table 1.

Table 1 Lifetime Prevalence Estimates of DSM-IV Nonaffective and Affective Psychoses

Diagnosis	N	LTP All	LTP Men	LTP Woman
Nonaffective psychotic disorders	153	1.94 (1.63-2.29)	1.64 (1.24-2.17)	2.19 (1.78-2.70)
Schizophrenia	67	0.87 (0.68-1.11)	0.82 (0.56-1.19)	0.91 (0.65-1.27)
Schizoaffective disorder	24	0.32 (0.21-0.46)	0.14 (0.06-0.34)*	0.47 (0.30-0.72)
Delusional disorder	15	0.18 (0.11-0.30)	0.16 (0.07-0.34)	0.21 (0.11-0.40)
Psychotic disorder NOS	38	0.45 (0.33-0.62)	0.33 (0.19-0.56)	0.56 (0.39-0.82)
All psychotic disorders	249	3.06 (2.66-3.51)	3.11 (2.54-3.57)	3.01 (2.54-3.57)

*This is a summary table of lifetime prevalence estimates based on population based survey of 8,028 persons 30 years or older screened for psychotic and bipolar I disorders from Perala et al., 2007. LTP = lifetime prevalence. Data are given as percentages and 95% confidence interval provided. Only nonaffective psychotic disorders with > 5 affected subjects detected are provided by diagnosis. 'All psychotic disorders' includes individuals with nonaffective psychotic disorders as well as those with affective psychoses, substance-induced psychotic disorder, and psychotic disorder to to a general medical condition. *Statistically significant difference ($p < 0.05$) between sexes detected.*

The age at onset of the disorder is early adulthood, with estimates ranging from about 18 – 24 years. There are detectable gender differences in various aspects of schizophrenia. The Perala et al study opposed the commonly held view that schizophrenia occurs equally frequently in males and females, instead documenting a 1.4:1 male:female ratio (Perala et al., 2007). Males have been shown in some studies to have a lower age at onset and more frequent occurrence of brain abnormalities whereas females generally have better premorbid functioning and less disability (Angermeyer & Kuhn, 1988; A. Jablensky et al., 1992; W.H.O., 1979). The decreased age at onset in seen in males has been replicable, and in some studies dramatic, but results have varied across studies (Hambrecht, Maurer, & Hafner, 1992; W.H.O., 1979) (see (A. Jablensky, 2000) for review). One plausible case of discrepancies between populations is the finding of a marked difference in sex ratio for late-onset schizophrenia (onset after age 40), which has been estimated at 1:1.9 male to female ratio after age 40 and up to 1:6 ratio after age 60 (Huber,

Gross, & Schuttler, 1979; A. Jablensky, 2000). It is therefore possible that difference in age at onset seen between males and females are a sampling artifact. For example, analysis of the more than 500 schizophrenia cases analyzed in this dissertation from Pittsburgh, PA, U.S. did not detect significant differences in age at onset of the disorder (unpublished data). However, subsets of cases were ascertained on the basis of family configurations, requiring both parents to participate as a case-parent trio, a sampling bias against ascertainment of late onset cases. Ascertainment criteria and study design can therefore have a significant impact on such analyses.

The environmental risk factors that have been associated with schizophrenia are too numerous and speculative to discuss at length here. Some of the more highly cited and replicated results across populations have included season of birth, maternal and / or paternal age, substance abuse, prenatal complications, comorbid medical conditions, viral infection, immune response, urban birth, urban versus rural residence, and immigration (see (A. Jablensky, 2000) for review). That there is an environmental influence in the etiology of schizophrenia seems certain based on the currently available data; however the ability to quantify the contribution of specific environmental factors on any given individual is limited. Therefore, to reduce the analytic space, the studies conducted herein consider environmental effects as an unknown confound and focus only on gender and age at onset of illness as potential covariates in the development of schizophrenia.

1.2.4 Treatment of schizophrenia

The first meaningful pharmacological success in the treatment of schizophrenia came with the introduction of chlorpromazine in the late 1950's. The therapy was extremely successful in reducing positive symptoms of schizophrenia, but not negative symptoms and cognitive deficits

often seen (Crow, 1980a, 1980b; Kane, 1990; Kane, Honigfeld, Singer, & Meltzer, 1988). Furthermore, the medication did not reduce symptoms in 5% - 25% of patients (Christison, Kirch, & Wyatt, 1991; Meltzer, 1992a, 1992b). About a decade later came the introduction of second generation, or atypical antipsychotics such as clozapine, olanzapine, risperidone, and others. While both generations of antipsychotics exert their influence on the dopaminergic system, specifically dopamine D_2 receptors, the atypical antipsychotics also affect the serotinergic system. These second generation agents were effective in reducing extrapyramidal side effects compared to typical antipsychotics, and were effective in the treatment of negative symptoms and cognitive deficits. The side effects of atypical antipsychotics include weight gain and metabolic effects. Atypical antipsychotics are significantly more expensive than typical antipsychotics, and recent clinical trials suggest atypical antipsychotics are not more effective than first generation agents and are not associated with better cognitive or social outcomes (Swartz et al., 2007). Nonetheless, the reduced extrapyrimidal side effects and improved reduction in negative symptoms still make atypical antipsychotics successful therapeutic agents in many cases. The current availability of first and second generation antipsychotics, as well as the newer 'third generation' antipsychotics, 'dopamine-serotonin stabilizers', give clinicians a range of options for patient specific therapy.

1.2.5 Schizophrenia pathogenesis: dopaminergic neurotransmission

Despite several decades of research and promising leads suggesting structural and functional neurological alterations, the pathogenesis of schizophrenia remains unknown (D. A. Lewis & Lieberman, 2000). The mechanism of antipsychotic agents, exerting their influence by binding targets in the central nervous system, suggests neurotransmitter dysfunction is a critical area for

study. Typical antipsychotics significantly reduce positive symptoms of schizophrenia by blocking dopamine receptors, whereas atypical antipsychotic include occupancy of serotonin receptors with consequent reduction in negative symptoms and cognitive deficits of the disorder. Neurotransmitter theories of schizophrenia have suggested multiple pathways, including glutamate and GABA alterations (Collier & Li, 2003). Yet the majority of evidence suggests dopamine is the final common pathway underlying psychotic symptoms, as well as negative and cognitive symptoms.

Dopaminergic neurons in the central nervous system project an extensive network of connections throughout the brain. Substantia nigra dopaminergic neurons project primarily to the striatum, and neurons in the ventral tegmental area project primarily to cortical (mesocortical pathway) and limbic (mesolimbic pathway) regions of the brain (Sillitoe & Vogel, 2008). The neostriatal dopaminergic pathway is thought to regulate motor control, while the mesocortical and mesolimbic pathways mediate many of the behavioral functions influenced by the dopaminergic system. These projections enable dopaminergic neurons to exert their diverse influence on a spectrum of behaviors from movement to cognitive function.

Hyperactivity of dopaminergic transmission was the first proposed 'dopamine hypothesis' of schizophrenia. The hypothesis broadly suggests that 'positive symptoms' result from hyperstimulation of D_2 receptors from hyperactive subcortical mesolimbic projections, and hypoactive mesocortical projections to the prefrontal cortex induce negative symptoms (Lang, Puls, Muller, Strutz-Seebohm, & Gallinat, 2007; Toda & Abi-Dargham, 2007). The hypothesis was initially supported by the correlations between the clinical potency of antipsychotics specifically on the D_2 receptors (Carlsson & Lindqvist, 1963; Creese, Burt, & Snyder, 1976; Crow, 1980a; Seeman, Lee, Chau-Wong, & Wong, 1976). It remains today that all effective

antipsychotics have some affinity for the D_2 receptor (Kapur & Mamo, 2003). Traditional antipsychotics bind tightly to the D_2 receptor with slow dissociation rates whereas atypical antipsycotics display faster dissociation rates, presumably accounting for the reduced extrapyramidal side effects (Seeman, 2002; Seeman & Tallerico, 1998, 1999). To test the hypothesis, a large number of variables have been investigated among cases and controls. They include post mortem dopamine receptor density, dopamine metabolite concentrations, *in vivo* measures of dopamine receptor density using PET scans, and the psychotogenic effects of agents that increase synaptic dopamine release (Abi-Dargham et al., 1998; Angrist & van Kammen, 1984; Breier et al., 1997; Davidson et al., 1987; Farde et al., 1987; Hess, Bracha, Kleinman, & Creese, 1987; Lieberman et al., 1984; Mackay et al., 1982; Seeman et al., 1987; D. F. Wong et al., 1986). Despite controversies, dopamine antagonism remains a key characteristic evaluated when novel agents are designed for schizophrenia (Davis, Kahn, Ko, & Davidson, 1991; D. A. Lewis & Lieberman, 2000). Recent evidence suggests that subtle dopamine dysregulation could occur in schizophrenia, rather than overall dopamine hyperactivity (Davis et al., 1991; Greene, 2006; Laruelle, Abi-Dargham, Gil, Kegeles, & Innis, 1999; Seeman et al., 2006). These subtleties likely reflect the impact of a number of susceptibility factors.

There is growing evidence for intricate homeostatic mechanisms that regulate dopamine homeostasis. The intensity and duration of dopamine signaling in the brain is determined by the amount of vesicular release, dopamine receptor sensitivity and the efficiency of dopamine clearance from the extracellular compartment (Gainetdinov, Sotnikova, & Caron, 2002; Torres, Gainetdinov, & Caron, 2003). Dopamine released into the synaptic space can undergo enzymatic degradation and dilution by diffusion. Two enzymes metabolize dopamine intracellularly, oxidative deamination by monoamine oxidase (MAO) and O-methylation by

COMT (Napolitano, Cesura, & Da Prada, 1995). However, the primary mechanism controlling extracellular dopamine levels has proven to be reuptake by presynaptic neurons via the plasma membrane dopamine transported (DAT) (Amara & Kuhar, 1993; Cragg & Rice, 2004; Giros & Caron, 1993). Thus, re-uptake through DAT is the most effective way to limit the lifetime of dopamine signaling in the brain.

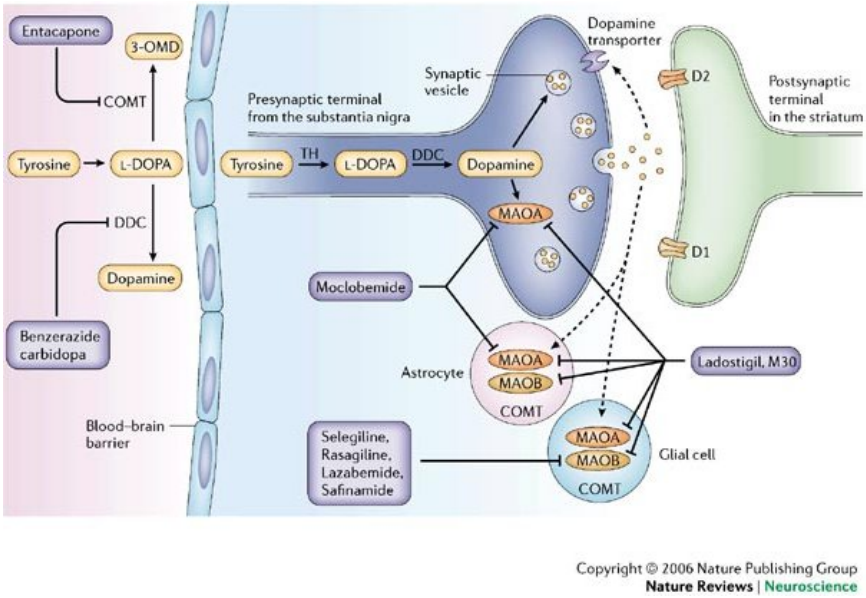

Figure 1 Key genes, localization, and function in the dopaminergic pathway
Figure from (Youdim, Edmondson, & Tipton, 2006). This figure shows the localization and action of several key dopaminergic genes investigated in this series of studies.

There are potential interactions that influence dopaminergic neurotransmission. Degradation of dopamine by COMT can influence the activity of DAT. The activity of DAT can also be regulated by dopamine autoreceptors. Both the DRD2 and DRD3 subtypes have been shown to be involved in the regulation of DAT function (Zahniser & Doolen, 2001). For example, the DRD3 receptor-preferred agonist PD 128907 produced an increase in DAT function in striatal slices as measured by rotating disk electrode voltametry (Zapata & Shippenberg, 2002) suggesting a cross-talk between the DRD3 and DAT. The molecular details of this cross-talk are not known, however these functional studies suggest important interactions

within the dopaminergic pathway could modulate dopamine transmission. The genes encoding dopaminergic proteins are further discussed in Chapter 3.0 below. One primary motivation for the genetic studies conducted herein is to determine if such functional interactions influence schizophrenia pathogenesis.

1.3 SCHIZOPHRENIA: A COMPLEX GENETIC DISORDER

1.3.1 Genetic epidemiology

Since its conception it has been observed that schizophrenia tends to cluster in families. The morbid risk in the general population is roughly 1%, however risk to children of schizophrenic probands is 13%. The morbid risk for monozygotic twins and offspring of dual patient mating are 48% and 46%, respectively, indicating a substantial genetic contribution (Figure 1) (I. Gottesman, 1991). Adoption studies also suggest a genetic basis for the disorder, finding that the familial aggregation cannot be explained solely by environmental influence (Heston, 1966; Kety & Ingraham, 1992). The sibling recurrence risk ratio, λs, is estimated at 8-10 (Risch, 1990). Heritability estimates have varied widely, ranging from 41-80% (Kendler & Robinette, ; McGue, Gottesman, & Rao, 1983; Rao, Morton, Gottesman, & Lew, 1981). Although controversial, particularly due to questions regarding clinical diagnoses (Farmer, McGuffin, & Gottesman, 1987; McGuffin, Farmer, Gottesman, Murray, & Reveley, 1984), recent review of 224 twin probands from the Maudsley Twin Register in London conducted after publication of the DSM-IV suggests the heritability of the disorder is on the high end of this distribution (Cardno et al., 2007). The authors derived estimates of heritability between 82% – 85%, with no significant

differences between diagnostic tools or diagnoses themselves in the full range of functional psychoses (schizophrenia, schizoaffective disorder, and mania). Similar estimates were derived from an earlier population based twin study in Finland (1180 male pairs and 1315 female pairs), which found that 83% of the variance in liability was due to additive genetic factors (Cannon, Kaprio, Lonnqvist, Huttunen, & Koskenvuo, 1998). Mode of inheritance is also unknown. Both autosomal dominant and recessive modes of inheritance have been suggested (Hurst, 1972; Slater, 1958). Complex segregation analyses of published family and twin data on the diagnostic trait suggest polygenic inheritance, likely including multiple genes of small effect (Carter & Chung, 1980; McGue et al., 1983; Rao et al., 1981). Regarding qualitative and quantitative traits associated with the disorder, a study currently in press found significant heritability and autosomal dominant inheritance for several endophenotypes (Aukes et al., 2008). It should be noted that the heritability of endophenotypic measures have not been shown to be higher than the diagnosis of schizophrenia itself (heritability range from 24% - 55% in various neurocognitive endophenotypes) (Greenwood et al., 2007). Based on these and similar studies over the last century, it is now widely accepted that the distribution of the disorder in families and populations is consistent with genetic models including multiple interacting loci of modest effect (Risch, 1990; Schliekelman & Slatkin, 2002).

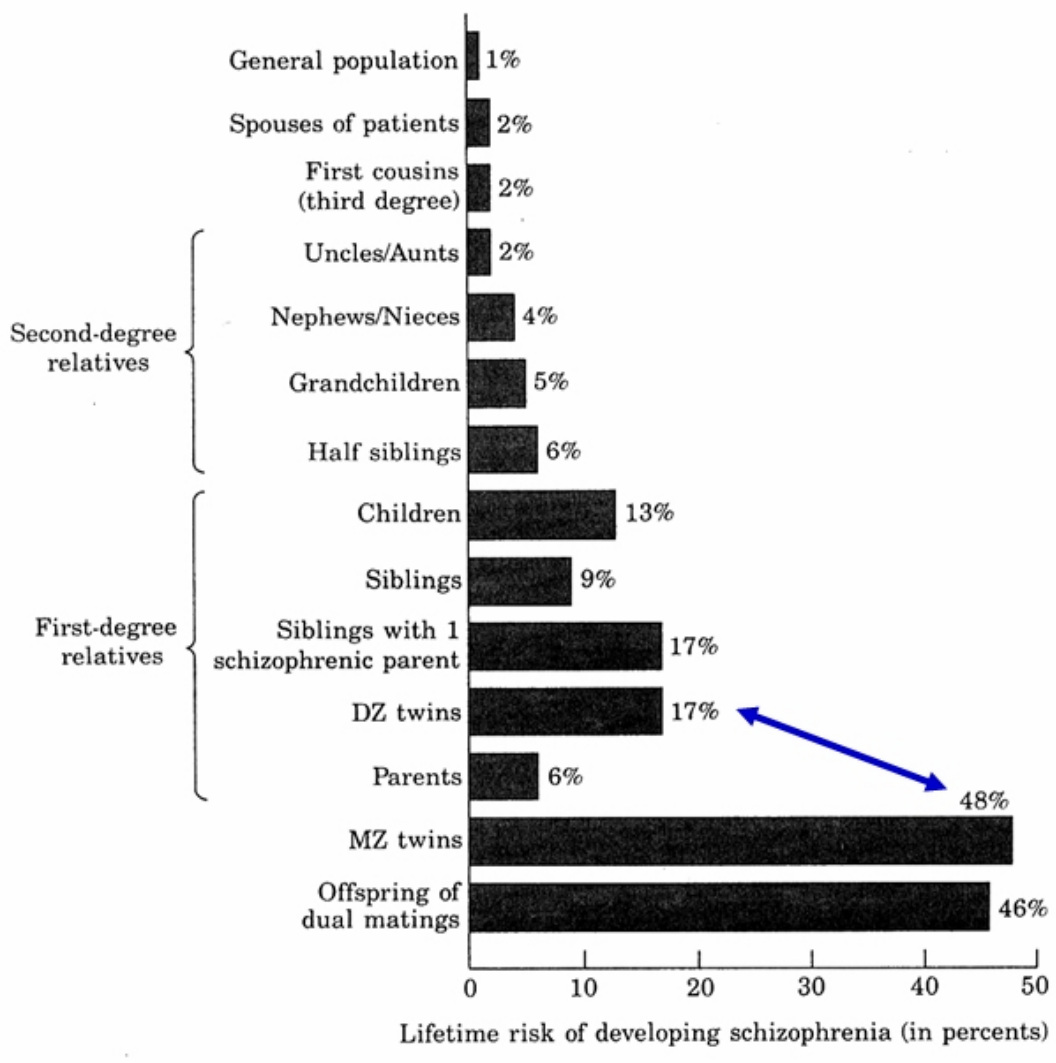

Figure 2 Morbid risks for schizophrenia

Lifetime risk of developing schizophrenia based on relationship to an affected individual. Values are given in percentages. Blue arrow shows discrepancy between monozygotic twins and dyzogotic twins. Figure adapted from Gottesman, 1991.

1.3.2 Linkage to schizophrenia

Genetic linkage is a test of the co-segregation within families of a phenotypic trait and a genetic locus. Linkage analyses have been extremely successful in mapping disease genes for many human diseases, including Huntington's disease, breast cancer, and cystic fibrosis, to name a

very few. The general strategy in gene mapping studies has been to first detect significant linkage, usually over a broad genomic region, then conduct focused fine mapping analyses to identify genes contributing to the linkage signal. Despite many documented successes of this 'positional cloning' method, the strategy has been relatively ineffective in psychiatric genetics. The potential reasons for this lack of success have been well documented and are likely due to limitations in power of most studies, presence of multiple disease genes of small effect, low penetrance, and a high degree of genetic heterogeneity between families. A large literature of linkage studies exists over the past two decades and putative linkage has been reported on nearly all autosomes and the X chromosome across populations, but there has been little consistency between studies. Initial studies were designed using a small number of extended pedigrees, a design ideally suited for identifying genes of large effect within families. One such strategy identified a "major susceptibility locus" on chromosome 1q21 – 22, with a reported LOD score of 6.50 (Brzustowicz, Hodgkinson, Chow, Honer, & Bassett, 2000). Yet a subsequent large scale, multi-site study with substantial power failed to confirm linkage in this region (Levinson et al., 2002). The more recent trend has been to incorporate large samples of smaller families in an effort to increase power to detect loci of small effect. A meta-analysis of the 20 largest linkage scans identified several regions of suggestive, but not significant, linkage to schizophrenia (C. M. Lewis et al., 2003). Subsequent analyses indicate these large studies could be more consistent than expected by chance (Zintzaras & Ioannidis, 2005), but it should be noted that the three largest sibling pair studies of schizophrenia in those analyses (> 300 sibling pairs per study) failed to detect overlapping linkage at a single locus in the genome (Crow, 2007).

1.3.3 Genetic association studies of schizohprenia

Unlike linkage, genetic association relies on linkage disequilibrium between a genetic marker and a disease locus over a relatively short genetic distance. When the frequency of a genetic marker is observed more / less frequently in a case sample than an unaffected comparison group at a level greater than one would expect by chance, the conclusion is genetic association with that variant or a correlated variation. The phenomenon is dependent on the presumption that the disease causing mutation occurred relatively recently, meaning the accumulation of recombination events within the population was insufficient to restore independence between the marker evaluated and the disease causing variant. There are several advantages of association studies compared to linkage; most notably the relative short genetic distance expected between actual liability locus and genetic marker, as well as the ability to accumulate large population based samples rather than the expense of ascertaining intact families.

The number of genetic association studies conducted on schizophrenia and related phenotypic traits over the last two decades are staggering. A PubMed search of "schizophrenia" "gene" and "association" retrieves 1,973 studies. According to a systematic meta-analysis and field synopsis of genetic association studies in schizophrenia (SzGene database), as of April 2007, 1,179 genetic association studies have been published worldwide reporting on 3,608 genetic variants from 516 different genes (Allen et al., 2008). The results of these studies have been largely inconsistent. Several promising targets, such as *DTNBP1* and *NRG1* have emerged, but in the estimation of this dissertation no robust genetic risk factors have been established from any individual genes. Despite these inconsistencies, reviews have suggested significant success in genetic epidemiological studies of schizophrenia, several of which are listed here (Harrison & Weinberger, 2005; (P. J. Harrison & Owen, 2003); (Craddock, O'Donovan, & Owen, 2005)

(Craddock, O'Donovan, & Owen, 2006) (Shirts & Nimgaonkar, 2004) (Owen, Williams, & O'Donovan, 2004), (Owen, Craddock, & O'Donovan, 2005); (Owen, Craddock, & Jablensky, 2007). The successes claimed in these reviews have been met with dissenting opinions, as Dr. Crow states "Thus this body of work must be regarded as an indicator of the extent to which the 'eye of faith' is able to discern meaning in complex data when none is present" (Crow, 2008b).

A detailed review of the literature regarding dopaminergic gene polymorphisms and schizophrenia pathogenesis are described in chapter two. The details of the remaining candidate genes reviewed in the articles listed above are too numerous and cumbersome to describe here, however it should be noted that many of these past genetic association studies followed a similar pattern. An initial study investigated very few variations (usually coding or putatively functional) for a single gene in a small sample. Replicate studies would then analyze only associated variants, leaving a sizeable gap in the literature regarding evaluation of representative genetic variation. A good example of this pattern can be seen in the investigative course of *RGS4*, where putative linkage and expression evidence lead to a significant association study with differing risk alleles and haplotypes between populations (Brzustowicz et al., 2000; Chowdari et al., 2002; Mirnics, Middleton, Stanwood, Lewis, & Levitt, 2001). No gene of large effect has subsequently identified to validate the linkage results, and the associations were proven to be false positive findings based on power and technological limitations in the initial reports, yet more than 25 samples have been studied to date since those findings. Nonetheless, analysis of over 13,000 individuals could neither support nor reject the null hypothesis of no association (Chowdari et al., 2007; Chowdari et al., 2002; Talkowski, Chowdari, Lewis, & Nimgaonkar, 2006; Talkowski, Seltman et al., 2006) (Talkowski et al., unpublished data).

Ambiguous results such as those at *RGS4* permeate the literature of schizophrenia association studies. The hypothesis underlying the series of studies presented in this dissertation is that any assumptions regarding these genes as schizophrenia susceptibility factors are premature and potentially erroneous. The empirical evidence obtained to date for most genetic association studies is insufficient to enable conclusions for, or against, credible risk factors. As simulations have shown, in the presence of an unmeasured liability locus, patterns of association can be complex amongst measured SNPs (Roeder, Bacanu, Sonpar, Zhang, & Devlin, 2005). This complexity obviously grows as the ratio of unmeasured SNPs to measured SNPs gets large, as is the case with most of these genes. Recent large scale efforts such as a study of 14 candidate genes by Sanders and colleagues (Sanders et al., 2008) and ongoing genomewide studies from the genetic analysis and information network (GAIN) initiative have begun to fill the void in the current literature and provide more reliable, if unspectacular, estimations of genetic risk conferred by susceptibility gene candidates.

2.0 DOPAMINE GENES AND SCHIZOPHRENIA: CASE CLOSED OR EVIDENCE PENDING?

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2.1 ABSTRACT

The dopamine hypothesis of schizophrenia has motivated a large number of genetic association studies, but few if any dopaminergic polymorphisms are accepted as credible risk factors at present. To evaluate whether dopamine related genes have been investigated adequately, we surveyed public genetic databases and published schizophrenia association studies with regard to fourteen conventional dopaminergic genes and seven selected dopamine interacting proteins. We estimate that 325 polymorphisms would be required to evaluate the impact of common variation on schizophrenia risk among Caucasian samples. To date, 98 polymorphisms have been analyzed in published association studies. We estimate that only 19 of these variations have been evaluated in samples with at least 50% power to detect an association of the effect size commonly found in genetically complex disorders. While it is possible that dopaminergic genes do not harbor genetic risk factors for schizophrenia, our review suggests that satisfactory conclusions for most genes cannot be drawn at present. Whole genome association studies have begun to fill this void, but additional analyses are likely to be needed. Recommendations for future association studies include analysis of adequately powered samples, judiciously selected polymorphisms, multiple ethnic groups and concurrent evaluation of function at associated SNPs.

2.2 INTRODUCTION

Over the past two decades schizophrenia (SZ) mapping studies have grappled with several difficulties inherent to all studies of common, genetically complex disorders. Heritability estimates for the disorder vary from 60-70% (McGue et al., 1983; Rao et al., 1981), but complex segregation analyses have consistently rejected monogenic models of inheritance in favor of polygenic / multi-factorial threshold models (Carter & Chung, 1980; McGue et al., 1983). A genetic model including multiple interacting loci of small effect may provide the best fit for the available data (Risch, 1990; Schliekelman & Slatkin, 2002; Sha, Zhu, Zuo, Cooper, & Zhang, 2006), making it difficult to identify individual genetic risk factors. Some analyses suggest that common genetic variants confer risk (also called the 'common variant common disease' hypothesis, CDCV) but others have argued in favor of rare variants (I. I. Gottesman, 1994; Jorde, 2000; McClellan, Susser, & King, 2007). Aided by technological and statistical advances, genetic association studies have grown in size and sophistication (Collins, Guyer, & Chakravarti, 1997; Hirschhorn, 2002). Thanks to these advances, some promising associations have been detected. For example, studies utilizing extended panels of single nucleotide polymorphisms (SNPs) have identified associations with polymorphisms of dysbindin (*DTNBP1*), neuregulin 1 (*NRG1*), disrupted in schizophrenia (*DISC1*), regulator of G protein signaling (*RGS4*), G72 and D-amino-acid oxidase (Craddock et al., 2005; P. J. Harrison & Weinberger, 2005; Owen et al., 2005; K. M. Prasad & Nimgaonkar, 2007). Consistent with the polygenic model, the risk conferred by the associated alleles is modest (odds ratios, OR ~1.2) (Shirts & Nimgaonkar, 2004)

A sizable fraction of other association studies have focused on dopaminergic genes, but few credible genetic risk factors have emerged. Two broad conclusions are thus possible: either

there are no significant associations between schizophrenia and dopamine polymorphisms or sufficient evidence is not currently available. In this review, we evaluate the possible impact of dopaminergic gene polymorphisms on schizophrenia risk. We summarize the motivation for, and details of, prior genetic association studies involving dopamine genes. We also survey public database information to determine the proportion of representative common variants that have actually been evaluated at these genes, and the number of SNPs analyzed with adequate power to detect an association of the modest effect sizes expected. We conclude with suggested designs for future studies and discuss the relevance of such studies in the context of whole genome association studies.

2.2.1 The dopamine hypothesis

The dopamine hypothesis suggests hyperactivity of dopamine brain function in schizophrenia pathogenesis. It originated from correlations between the clinical potency of anti-psychotic drugs and their affinity for dopamine D2 receptors (DRD2) (Carlsson & Lindqvist, 1963; Creese et al., 1976; Seeman et al., 1976). Two lines of enquiry have yielded relatively consistent results regarding the dopamine hypothesis of schizophrenia. First, patients with schizophrenia display increased sensitivity to the psychotogenic effects of agents that increase synaptic dopamine release (Angrist & van Kammen, 1984); Lieberman, 1984 #2673; Davidson, 1987 #2672; Laruelle, 1999 #2664}. Second, acute amphetamine challenge to patients leads to increased dopamine transmission *in vivo*, as measured by radioligand binding to dopamine D2 receptors during positron emission tomography (PET) scans (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). However, the dopamine hypothesis has not been supported consistently using measures such as *post mortem* dopamine receptor density or dopamine

metabolite concentrations, *in vivo* measures of dopamine receptor density using PET scans or dopamine metabolite concentrations in the cerebrospinal fluid (Bird et al., 1977; Cross, Crow, & Owen, 1981; Farde et al., 1987; Hess et al., 1987; Mackay et al., 1982; Seeman et al., 1987; Widerlov, 1988; D. F. Wong et al., 1986). The discrepancies could be due to medication effects and sampling variation (Davis et al., 1991; D. A. Lewis & Lieberman, 2000).

Refining the dopamine hypothesis: Subtle dopamine dysregulation could occur in schizophrenia, rather than overall dopamine hyperactivity; e.g., regional variation, selected receptor types, temporal sensitization or variations during different phases of illness (Davis et al., 1991; Greene, 2006; Laruelle et al., 1999; Seeman et al., 2006). Hypofunction in prefrontal neuronal circuits has been documented repeatedly in post-mortem brain studies of schizophrenia; this may also lead to disinhibition of the prefrontal drive to the limbic striatum with a resultant hyperdopaminergic state in the limbic striatum (D. A. Lewis & Lieberman, 2000; Weinberger, 1987). These subtle changes likely reflect a chain of events, so a number of susceptibility factors may be present. This is consistent with the polygenic model of schizophrenia.

2.2.2 Genetic association studies using dopamine polymorphisms

The extensive interest in the dopamine hypothesis has also motivated numerous association studies of dopamine genes under the rationale that credible genetic associations would motivate further studies of pathogenesis. However, most early association studies were hampered by significant deficiencies in technology and relatively modest sample sizes available. Despite these limitations, the gamut of genes involved in dopamine neurotransmission was investigated. We conducted PubMed searches using the following combinations of terms: (1)

"(individual gene name)" and "schizophrenia"; (2) "(gene symbol)" and "schizophrenia"; (3) "dopamine" and "schizophrenia". Genetic association studies were then extracted from these sets. As discussed below, most studies followed a similar pattern. An initial study reported on one or a few putatively functional polymorphisms and subsequent studies analyzed only those variants. Some study designs, such as mutation detection followed by association tests in relatively small samples, are better suited to identify susceptibility loci harboring a substantial impact on risk. Thus, no consistent associations have been detected for a number of key dopaminergic genes, potentially leading to the conclusion that susceptibility variants are not present in the dopaminergic network.

The dopaminergic genes investigated in multiple independent samples include tyrosine hydroxylase (*TH*) (Chao & Richardson, 2002; Ishiguro, Arinami et al., 1998; D. Li & He, 2006), dopamine decarboxylase (*DDC*) (Borglum et al., 2001; Zhang et al., 2004), dopamine beta hydroxylase (*DBH*) (Cubells & Zabetian, 2004; Jonsson, Abou Jamra et al., 2003; Tang et al., 2006; Yamamoto et al., 2003), *COMT* (see below), *MAOA* (Jonsson, Norton et al., 2003; Nolan, Volavka, Lachman, & Saito, 2000; Norton et al., 2002; Sabol, Hu, & Hamer, 1998; Syagailo et al., 2001; Tunbridge, Harrison, & Weinberger, 2006), and one of the two isoforms of the vesicular monoamine transporter (*SLC18A1*, alias VMAT1) (Bly, 2005; S. F. Chen et al., 2007; Richards et al., 2006). The dopamine receptors *DRD1*, *DRD2*, *DRD3*, *DRD4*, and *DRD5* have also been investigated (Cichon et al., 1996; Fanous et al., 2004; Glatt & Jonsson, 2006; Jonsson, Kaiser, Brockmoller, Nimgaonkar, & Crocq, 2004; Muir et al., 2001; A. H. Wong, Buckle, & Van Tol, 2000). The vesicular monoamine transporter, member 2 (VMAT2, *SLC18A2*) has only been investigated in one study to date (Kunugi, Ishida, Akahane, & Nanko, 2001).

Other investigators have reported on dopamine interacting proteins, with similarly inconsistent results. They include Orphan Nuclear Receptor Subunit 4 (NURR, *NR4A21*); D1 Receptor Interacting Protein (CALCYON, *DRD1IP*); Protein Phosphatase 1, Regulatory (inhibitory) subunit 1B (dopamineRPP-32, *PPP1R1B*); Syntaxin 1A (*STX1A*); Protein Interacting with PRKCA 1 (*PICK1*); Synaptosomal-Associated Protein, 25kDa (*SNAP25*); and Beta Adrenergic Receptor Kinase 2 (GRK3, *ADRBK2*) (Y. H. Chen, Tsai, Shaw, & Chen, 2001; Fujii et al., 2006; Hong, Liao, Shih, & Tsai, 2004; Ishiguro et al., 2007; Iwayama-Shigeno et al., 2003; C. H. Li, Liao, Hung, & Chen, 2006; Luo et al., 2004; Tachikawa, Harada, Kawanishi, Okubo, & Suzuki, 2001; A. H. Wong et al., 2004; S. Y. Yu et al., 2004).

Since space restrictions preclude detailed discussion of each gene, we have reviewed four of the most extensively analyzed dopamine genes. While early association studies have been inconsistent for all of them, studies published in the past decade have provided intriguing new facets. Each gene thus provides precepts for future association studies.

2.2.3 Dopamine D2 receptor (*DRD2*)

The dopamine D2 receptor was a logical early target for association studies because of the effects of therapeutic agents reviewed above. Two genetic variants have been the target of most studies. One is a cysteine to serine substitution at codon 311 (Cys311Ser); the other an insertion / deletion 141 bases in the 5' region of the gene (-141C ins/del). Two independent meta-analyses identified a significant association between the rare Cys311 allele and schizophrenia (Glatt, Faraone, & Tsuang, 2003b; Jonsson, Sillen et al., 2003), a result that has since been confirmed by a more comprehensive meta-analysis including data from 3,707 cases and 5,363 controls (Glatt & Jonsson, 2006). In contrast, a meta-analysis did not support an association with the

insertion / deletion polymorphism. Other polymorphisms have been investigated more recently with significant results from four different studies (Dubertret et al., 2004; Hanninen et al., 2006; Kukreti et al., 2006; Parsons et al., 2007) but significant associations were not detected when 5 SNPs were analyzed among a family cohort of Askhenazi Jewish families (M. D. Fallin et al., 2005). It would be instructive if the same set of polymorphisms could be analyzed in all these samples, followed by meta-analysis.

2.2.4 Dopamine D3 receptor (*DRD3*)

Over 50 studies have sought associations at *DRD3*, but most have focused exclusively on rs6280 (Ser9Gly), a non-synonymous SNP in the first exon with possible functional effects (Jeanneteau et al., 2006). Repeated meta-analyses have suggested a modest association, but all meta-analyses have not been consistent (Ioannidis, Ntzani, Trikalinos, & Contopoulos-Ioannidis, 2001; Jonsson et al., 2004; Shaikh et al., 1996). Recent studies have evaluated other variations with somewhat more consistent results. Four studies focused on associations with SNPs upstream to exon 1 (Anney et al., 2002; Ishiguro, Ohtsuki et al., 1998; Sivagnanasundaram et al., 2000; Staddon et al., 2005). Three of these studies detected significant associations, suggesting inconsistencies at rs6280 could represent associations with other, correlated SNPs. However, one large case-control study and analysis of a family based sample did not reveal any significant associations (M. D. Fallin et al., 2005). Two recent studies evaluated a larger proportion of representative variation; both detected significant haplotype based associations. We found significant associations with SNPs and haplotypes spanning the gene in two independent samples (Talkowski, Mansour et al., 2006). Another group reported significant haplotype based associations in the 3' region of *DRD3* in a Galician population (Dominguez et al., 2007). In

sum, the numerous association studies conducted at rs6280 appear to be equivocal with respect to schizophrenia susceptibility; however more recent results considering a greater proportion of common variation within the gene have been more encouraging. These recent findings may represent other liability loci at this gene and might highlight the value of comparative analyses of varied ethnic groups. Such studies lend themselves to evolutionary analyses that may identify ancient mutations (Seltman, Roeder, & Devlin, 2003; Templeton, Boerwinkle, & Sing, 1987; Templeton, Weiss, Nickerson, Boerwinkle, & Sing, 2000).

2.2.5 Catechol-o-methyltransferase (*COMT*)

COMT is localized to chromosome 22q11, a region implicated in several linkage studies (C. M. Lewis et al., 2003). Deletions in this region also lead to the velocardiofacial syndrome, with an increased risk of psychoses (Karayiorgou et al., 1995). Most association studies have investigated an exonic Met158Val polymorphism, which appears to influence COMT activity in vitro. Two different meta-analyses suggest that an association between this variant and schizophrenia, if present, is complex and may be influenced by population substructure (Glatt, Faraone, & Tsuang, 2003a; Lohmueller, 2003). Interest in the Met158Val polymorphism has continued because it may be correlated with working memory, a trait known to be impaired in schizophrenia (Barnett, Jones, Robbins, & Muller, 2007; Egan et al., 2001; Tunbridge et al., 2006).

Recent association studies have investigated a larger set of SNPs. Li examined eight markers in a Chinese sample and detected a significant association with an extended haplotype including Met158Val (T. Li et al., 2000). Another large study of Ashkenazi Jewish patients revealed a highly significant association with two COMT SNPs, as well as a haplotype comprising 3 SNPs

spanning the 5' to 3' region of the gene (rs737865–rs4680–rs165599) (Shifman et al., 2002). However, a study among unrelated cases and controls did not replicate this finding (H. J. Williams et al., 2005), nor did a study of 274 Ashkenazi families investigating 7 COMT SNPs (M. D. Fallin et al., 2005). Intriguingly, The Met158Val polymorphism was part of this haplotype and the association was more prominent among women. Gender specific associations have been detected with a variant within this haplotype (rs737865) in Alzheimer's disease as well (Sweet et al., 2005). Notably, rs737865 is in proximity to an estrogen response element (Sweet et al., 2005). These associations highlight the need to evaluate valid sub-groups of schizophrenia and the need to consider functional impacts of associated alleles.

2.2.6 Dopamine transporter (*DAT, DAT1, SLC6A3*)

Most association studies have focused on a functional tandem repeat (VNTR), 3' to the stop codon in exon 15, but meta-analyses suggest no significant association (Fanous et al., 2004; Gamma, Faraone, Glatt, Yeh, & Tsuang, 2005; Mitchell et al., 2000; Vandenbergh et al., 1992). An association has been reported with an exonic SNP among Koreans (1389 C>T; rs2270912) (Jeong, Joo, Ahn, & Kim, 2004). A case-control study among Iranians identified a significant association with a putative promoter variant (-67A/T; rs2975226; $p = 0.0003$; OR = 2.25) (Khodayari et al., 2004). The association is particularly intriguing because *cis*-acting variation in the 5' region of this locus may contribute to differential *SLC6A3* expression *in vitro* and *in vivo* (Drgon et al., 2006; Kelada et al., 2005). The Korean and Iranian studies need to be evaluated in additional samples. Additional studies using common polymorphisms spanning the gene are also required.

2.3 PUBLISHED DOPAMINE ASSOCIATION STUDIES

We examined 14 dopamine genes and 7 dopamine interacting proteins that have been used for prior association studies. Our goal was to identify a representative set of common SNPs that should be evaluated to enable a reasonable test of the CDCV hypothesis for each gene. The samples utilized were 60 unrelated Caucasians from the International HapMap project (CEPH population) (HapMap, 2003) or 90 unrelated individuals representative of the US population from the NIH Polymorphism Discovery Resource 90 individual subset (PDR90) (http://egp.gs.washington.edu/). Data was obtained using the Genome Variation Server resource (http://gvs.gs.washington.edu/GVS/) (Carlson et al., 2004). All SNPs with minor allele frequencies over 5% were identified, since currently available samples may lack power to detect associations with less frequent polymorphisms. Since genotypes at many of these SNPs may be correlated due to linkage disequilibrium (LD), we selected representative 'tag' SNPs using a conventional cutoff ($r^2 < 0.8$ between loci). Based on these analyses, we found that 325 tag SNPs would be needed to tag all available common variations from these populations (Table 2).

These estimates were next compared with the published association studies. At each gene, we listed the number of variations evaluated in previous association studies (SNPs and other polymorphisms), as well as the largest individual association study for each gene (defined in terms of the number of cases, see Table 2). If possible, LD between the polymorphisms was analyzed. We also estimated the number of studies that had 50% power to detect associations of modest effect size for each of the polymorphisms tested (alpha = 0.05). We assumed an additive risk model with a genotype relative risk of 1.5 for homozygous individuals, 1.25 for heterozygous individuals, and a disease prevalence of 1%. We also assumed that the marker

being considered was the actual liability variant and that genotyping errors were negligible. Thus, our power estimates are relatively lax.

Ninety eight different polymorphisms have been investigated in all the association studies to date. We find that only *DRD5* has been comprehensively covered when considering the proportion of representative variations genotyped and power (Table 2). If each of the published polymorphisms represents a tag SNP, 30.1% of the required tag SNPs may have been evaluated. In reality, the proportion of representative SNPs analyzed in the publications is almost certainly lower, since we were unable to estimate LD between many of these polymorphisms and several rare polymorphisms have been analyzed (data not shown). We estimate that 19 of the polymorphisms studied had greater than 50% power to detect a genotype relative risk expected at an alpha threshold of 0.05. Thus, most of the published studies, including those reporting on the genes with extensive numbers of polymorphisms are likely to lack sufficient power, even using our relaxed criteria. Under more realistic conditions (D' = 0.9 between the genotyped marker and liability locus, 0.5% error rate, 1:1 case/control ratio, and a risk allele frequency of 0.2), we estimate that 595 cases and 595 controls would be required for 50% power under an additive model and 275 cases / 275 controls would be required under a dominant model of inheritance (1217 cases and 561 cases, respectively, would be required for 80% power under each model) (Purcell, Cherny, & Sham, 2003; Sham, Cherny, Purcell, & Hewitt, 2000). These estimates are with regard to single marker analysis. Additional corrections would be required for multiple independent tests. Since analyses of epistatic interactions would require further corrections for multiple comparisons, the sample size requirements for identifying such effects will be even larger.

2.4 SUGGESTIONS FOR FUTURE ANALYSES

2.4.1 Are more genetic association studies needed?

Given the difficulties outlined above, it is worthwhile to weigh the utility of further gene mapping studies for schizophrenia. We believe such studies are needed, primarily because it has been difficult to pinpoint environmental risk factors reliably (A. Jablensky, 1997; A. V. Jablensky & Kalaydjieva, 2003). Gene mapping studies have been recommended for such disorders, particularly if they have substantial heritability (Merikangas & Risch, 2003). The substantial body of evidence pointing to dopamine dysfunction in schizophrenia is a natural starting point to re-evaluate available evidence.

Some may argue against the need for further dopamine genetic studies because dopamine function is already an area of intensive research, including drug development efforts. However, genetic association studies may provide additional value for such research. First, emerging evidence suggests that networks of functionally related genes may be involved in pathogenesis of many multi-factorial disorders (Vogelstein, Lane, & Levine, 2000). Carefully designed genetic studies might enable the identification of such networks, including key nodes to which novel therapeutics can be targeted (Goh et al., 2007). Second, such analyses might help identify novel genes related functionally to 'conventional' dopamine genes.

2.4.2 Which genes should be targeted?

Apart from the genes involved in dopamine metabolism or those encoding dopamine receptors, a definition of 'dopamine' genes is difficult, because of the known cross-talk between

neurotransmitter systems. Any list of 'dopamine genes' is also unlikely to remain static in the face of advances in neuroscience research. We recommend starting with genes for which prior association evidence is available. If further studies provide credible, consistent associations, additional functional interactants of the associated genes can be targeted.

2.4.3 Which polymorphisms should be investigated?

Different types of polymorphisms are known in the human genome, ranging from SNPs to large copy number variations (CNVs) (Fanciulli et al., 2007). SNPs are obvious starting points because they have been characterized extensively and because they can be assayed cheaply and accurately. A secondary question is the choice of SNPs. While it is relatively easy to select representative tag SNPs, the allele frequency of the selected SNPs is a more difficult choice. The feasibility of detecting associations for common diseases using 'common' SNPs has been questioned on the grounds that they may not mirror the primary associations accurately and / or because risk may be due to relatively rare alleles (McClellan et al., 2007; Moskvina & O'Donovan, 2007; Terwilliger & Goring, 2000; Terwilliger & Hiekkalinna, 2006).

While the possibility of rare variants predisposing to schizophrenia can not be discounted, currently available samples may not enable detection of statistical associations if such variants are examined directly. One practical solution may be to select common tag SNPs, and follow up suggestive associations with more dense sets of SNPs, including rare variants. Such intensive analyses may enable us to detect causal variants.

Table 2 Published dopaminergic gene association studies and estimates of coverage

				Publicly Available SNP Data[1]		Published Studies[2]		Largest Study*	Meta-Analyses[3]	
Gene	**Location**	**Gene Name *(alias name)***	**Size (kb)[4]**	Common SNPs (MAF> 5%)	Tag SNPs[5]	# Markers Studied[6]	Power > 50%[7]	Cases / Controls **	SNPs	Result
Dopamine Pathway Genes										
TH	11p15.5	Tyrosine hydroxylase	17.9	14	10	2	1	334/391	1	-
DBH	9q34	Dopamine beta hydroxylase	33	68	39	2	0	178/178		
DDC	7p11	Dopamine decarboxylase	112.6	204	36	2	0	173/204		
DRD1	5q35.1	Dopamine D1 receptor	13.1	12	7	2	1	407/399		
DRD2	11q23*	Dopamine D2 receptor	75.6	78	19	7	0	-274	2	+/-
DRD3	3q13.3	Dopamine D3 receptor	60.2	69	18	17	4	331/280, (291)	1	+
DRD4	11p15.5	Dopamine D4 receptor	13.4	4	2	5	5	630/520	2	-
DRD5	4p16.1	Dopamine D5 receptor	12.1	1	1	2	0	158/437		
SLC18A1	8p21.3*	Vesicular monoamine transporter, member 1 (*VMAT1*)	48.4	60	20	4	0	354/365		
SLC18A2	10q25	Vesicular monoamine transporter, member 2 (*VMAT2*)	45.9	43	15	6	0	50		
SLC6A3	*5p15.3*	neurotransmitter transporter, dopamine *(DAT, DAT1)*	*62.6*	120	49	7	0	252/271	1	-
COMT	*22q11.2**	Catechol-O-methyltransferase	*37.2*	50	30	11	3	1643/3980	1	+/-
MAOA	Xp11.3	Monoamine oxidase A	100.7	38	8	3		346/334		
MAOB	Xp11.3	Monoamine oxidase B	125.8	16	12	0	0			
Dopamine Interacting Genes										
NR4A2	2q24.1*	orphan nuclear receptor subunit 4 (*NURR1*)	18.3	6	3	2	0	180/180		
DRD1IP	10q26.3	D1 receptor interacting protein (*CALCYON)*	21.5	5	4	1	0	276/253		
PPP1R1B	17q21.2	protein phosphatase 1, regulatory (inhibitory) subunit 1B *(DARPP-32)*	19.7	3	1	3	0	249/273		
STX1A	7q11.23	Syntaxin 1A	30.4	7	3	4		192/192, (238)		

Table 2. Continued

PICK1	22q13.1	Protein interacting with PRKCA 1	28.4	17	6	3		1765/1851
SNAP25	20p12-p11.2	Synaptosomal-associated protein, 25kDa	98.5	97	32	1	0	87/100
ADRBK2	22q12.1	beta adrenergic receptor kinase 2 *(GRK3)*	159.9	10	10	14	0	(16) and (97)

*[1]Publicly available genotype data: HapMap build 36 (www.Hapmap.org) (Thorisson, Smith, Krishnan, & Stein, 2005), and the NIHPDR 90 screening subset (http://gvs.gs.washington.edu/GVS/index.jsp). [2]Data from PubMed searches, see details in the text. [3]Number of SNPs at which meta-analysis has been conducted is provided. (+) = significant association detected, (-) = no significant association, (+/-) = conflicting results among meta-analyses. Blank spaces indicate that meta-analyses have not been published. [4]Includes sequences 5 kb upstream (5') and 5 kb downstream (3') of the gene. [5] Tag SNPs selected as described in the text. Repeat polymorphisms not included. [6] Indicates number of studied polymorphisms that were not redundant ($r^2 < 0.8$, where feasible). [7]Number of SNPs for which individual study evaluating the SNP had 50% or greater power to detect an association; see details in the text. [8]Study included samples from the US (151 trios, 331 cases, 274 controls) and India (141 trios) [9]Study analyzed 16 Japanese families and 97 Chinese families. *Studies with largest number of cases are included. ** Where family based samples were used, the number of families is listed in brackets.*

2.4.4 Sample Configurations

The possibility of spurious associations due to ethnic admixture has motivated much debate and the espousal of family based association studies (Spielman & Ewens, 1993; Wacholder, Rothman, & Caporaso, 2000). While family based samples detect association only in the presence of linkage and are thus particularly valuable, it is now feasible to correct for population sub-structure (Bacanu, Devlin, & Roeder, 2000; Pritchard & Rosenberg, 1999; Spielman & Ewens, 1993). Though the choice of controls may be dictated by convenience, biased selection of controls has obvious implication for detecting associations. Hence it is important to plan for follow up initial associations in other independent samples.

2.4.4.1 Sample size

The power analyses reviewed above suggest the need for relatively large samples. Given the possibility of false positive associations, replicate analyses are also recommended (Editorial, 1999). While sample size limitations remain significant hurdles for association studies, the availability of public repositories (http://www.nimh.nih.gov/), and the feasibility of staged analyses (Skol, Scott, Abecasis, & Boehnke, 2006) may make this issue more tractable.

2.4.4.2 Which ethnic group/s?

The overwhelming majority of genetic association studies are being conducted among individuals of Caucasian ancestry. Our review suggests ethnic variation in the magnitude

of some of the associations. Such variation is known in other disorders, for example the association between ApoE alleles and Alzheimer disease (Farrer et al., 1997). Evaluation of multiple ethnic groups may also enable us to identify primary associations based on ancestral recombinations (Templeton et al., 2000).

2.4.4.3 Functional Analysis

The majority of genetic associations for schizophrenia have been reported with non-coding polymorphisms, making it difficult to attribute function to the associated alleles. Nevertheless, such analyses are critical for understanding pathogenesis and may also be helpful in determining primary associations. An interactive design, with genetic associations informing functional analyses, and *vice versa*, is desirable.

2.4.4.4 Should genomewide associations (GWAS) supplant candidate gene studies?

Recently, GWAS have come to the fore, thanks to the availability of a comprehensive trove of common polymorphisms, rapid and accurate genotyping platforms and sophisticated analytic techniques. By analyzing a representative set of SNPs among cases and controls, GWAS studies seek to evaluate the relative impact of common polymorphisms. Judicious analyses may also provide insights into epistatic interactions. Remarkable consistencies have recently been attained for a diverse set of common diseases, including age related macular degeneration, prostate cancer, Crohn's disease and type I diabetes mellitus (Ennis et al., 2007; Gudmundsson et al., 2007; Libioulle et al., 2007; Saxena et al., 2007). GWAS studies have already been reported for schizophrenia (Lencz et al., 2007) and other independent studies are in progress. These

studies are likely to yield important new insights, so it is reasonable to question the need for focused candidate gene studies.

It is important to note that GWAS represent the beginning of a new effort, rather than an end point in the gene mapping effort. For example, GWAS studies will undoubtedly require replicate studies, followed by more detailed analysis of prioritized genes using more dense sets of polymorphisms. Thus, 'candidate gene analyses' will still be needed. Indeed, common polymorphisms are not tagged uniformly across the genome in some arrays used for GWAS. Thus, key associations may remain undetected, even with GWAS. In other diseases, candidate gene analyses have also identified associations with SNPs that were not sufficiently large for detection using GWAS; e.g., associations between late-onset Alzheimer disease and *SORL1* SNPs (Rogaeva et al., 2007).

2.5 CONCLUSIONS

Our review of published association studies involving dopaminergic genes highlights the lack of adequate analyses of variation at these genes. Our findings suggest more comprehensive analyses are required in sufficiently powered samples, particularly in view of some promising recent results. Replicate analyses, as well as analyses of multiple ethnic groups, in conjunction with functional evaluation of associated SNPs would be preferable.

2.6 ACKNOWLEDGEMENTS

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3.0 STUDY #1: NOVEL, REPLICATED ASSOCIATIONS BETWEEN DOPAMINE D3 RECEPTOR POLYMORPHISMS AND SCHIZOPHRENIA

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Novel, replicated associations between dopamine D3 receptor gene polymorphisms and schizophrenia in two independent samples. Biol Psychiatry. 2006 Sep 15;60(6):570-7.

3.1 ABSTRACT

Meta-analyses have suggested an association between schizophrenia and a coding polymorphism (rs6280/Ser^{9}Gly) at the dopamine D3 receptor gene (*DRD3*), but results have been inconsistent. Since most studies have evaluated only rs6280, the inconsistencies may reflect associations with other variants. We analyzed 13 polymorphisms spanning 109kb in two independent samples (US: 331 cases, 151 trios, 274 controls; India: 141 trios). In the U.S. samples, significant associations were detected with eight SNPs, including rs6280 (p = 0.001, OR:1.5, 95% CI:1.2-1.9). Consistent associations in the case-control and family-based analyses were detected with a common haplotype spanning intron 1 to the 3' region of the gene (rs324029-rs7625282-rs324030-rs2134655-rs10934254; case-control, p=0.002, TDT, p=0.0009; global p-values = 0.002 and 0.007, respectively). In the Indian sample, one SNP was associated (rs10934254, p=0.03). Moreover, over-transmission of the same common haplotype as the U.S. sample was observed in this cohort (TDT, p=0.005; global test, p=0.009). Ser^{9}Gly (rs6280) was associated with schizophrenia against this haplotype background, but not other haplotypes.

These data suggest inconsistent findings at rs6280 may result from associations with other *DRD3* variants. A liability locus may be in LD with, or carried against, an associated haplotype spanning the gene. Comprehensive SNP evaluation in larger samples is needed.

3.2 INTRODUCTION

Dysfunction in the dopamine D3 receptor (*DRD3*) has long been implicated in the pathogenesis of schizophrenia (see (Gingrich & Caron, 1993); (Sokoloff & Schwartz, 1995) for review). *DRD3* mRNA is predominantly expressed in the limbic system, a region thought to be dysfunctional in schizophrenia (Suzuki, Hurd, Sokoloff, Schwartz, & Sedvall, 1998). Indeed, increased *DRD3* receptor density has been noted in the mesolimbic region of post-mortem brain samples from patients with schizophrenia (A. M. Murray, Ryoo, Gurevich, & Joyce, 1994). Post-mortem studies have also revealed decreased levels of *DRD3* mRNA in cortical regions (Schmauss, Haroutunian, Davis, & Davidson, 1993). These changes may be pathogenic, since D3 receptors are thought to mediate antipsychotic drug action (Sokoloff et al., 1992) (Schwartz, Diaz, Pilon, & Sokoloff, 2000).

DRD3 maps to chromosome 3q13.3. Within the gene there is a common, non-synonymous coding polymorphism in exon 1. The single base change codes for either serine or glycine at the ninth amino acid in the N-terminal extracellular domain ($Ser^{9}Gly$; rs6280) (A. H. Wong et al., 2000). Genotypes of this variant have been reported to show differential affinity for dopamine (Lundstrom & Turpin, 1996), rendering it an intriguing functional candidate polymorphism. Since the initial study reporting an association with schizophrenia (Crocq et al., 1992), this polymorphism has been among the most extensively investigated variants in psychiatric genetics.

Consistent associations with schizophrenia have been sought at this locus in over forty samples to date, the majority involving case-control designs (see (Jonsson, Flyckt et al., 2003). Associations have been reported and replicated with increased homozygosity, as

well as the serine allele (rs6280 allele A; often reported as allele 1). However, a number of studies have not replicated these results. These data have motivated multiple meta-analyses (Nimgaonkar et al., 1996) (Shaikh et al., 1996) (J. Williams et al., 1998) (Dubertret et al., 1998) (Jonsson, Flyckt et al., 2003) (Jonsson et al., 2004). Meta-analyses were conducted successively, and in sum they appeared to suggest a significant, but modest association with the serine variant (n = 8,761; estimated OR = 1.10, 95% CI = 1.01 – 1.20) (Jonsson, Flyckt et al., 2003). However, the most recent and largest effort to evaluate this polymorphism did not detect significant associations with schizophrenia (n = 11,066; Jonsson et al. 2004).

Thus, despite an impressive compilation of data across multiple populations, the impact of this variant in schizophrenia pathogenesis has remained inconclusive. There are several explanations for inconsistent results apart from stochastic variation. One possibility is that the $Ser^{9}Gly$ variant itself is not associated with schizophrenia, but is in modest linkage disequilibrium (LD) with an unidentified liability locus. Such a scenario could produce inconsistent associations similar to those previously reported. Several investigators have attempted to address this hypothesis, focusing primarily on exon 1 and the immediate 5' region of the gene. Three studies have reported associations with polymorphisms spanning approximately 7 kb 5' to exon 1 (Ishiguro, Ohtsuki et al., 1998) (Sivagnanasundaram et al., 2000) (Staddon et al., 2005). In contrast, Anney and colleagues failed to detect associations in this region following mutation screens and analysis of 736 Caucasian cases and controls (Anney et al., 2002). Asherson et al. screened all exons and regulatory regions at *DRD3* in a small number of cases and controls (36 cases, 36 controls) and found two variations, one at exon 3 and a 5 bp

deletion in the 3' intron flanking exon 5. Neither of these mutations alter the protein structure, nor were they associated with schizophrenia in this study (Asherson et al., 1996).

Given the considerable number of positive, albeit inconsistent associations reported, results of these past studies may be reflecting an association between schizophrenia and another variant at *DRD3*. As available databases such as the International Hapmap Project reveal SNPs within and flanking the gene are not in strong linkage disequilibrium with rs6280, it appears that other variants at this locus have not been accounted for in the current literature. We report here analyses of 13 polymorphisms spanning the *DRD3* gene and flanking regions (109 kb) in two independent samples using both case-control and family-based designs. We also evaluate coverage of this and past studies through linkage disequilibrium analyses using all publicly available genotype data across this region.

3.3 METHODS

3.3.1 Clinical

Recruitment of probands was performed in Pittsburgh and surrounding regions for the U.S. sample (n = 331 cases), as well as New Delhi and surrounding regions for the Indian sample (n = 141 cases). In a concerted effort to limit heterogeneity between populations, both samples were ascertained using identical criteria. All participants completed a semi-structured interview (Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994).

In addition, supplemental information was obtained from medical records and relative reports as required for consensus diagnosis by board certified psychiatrists / psychologists. All cases were diagnosed with schizophrenia or schizoaffective disorder (DSM-IV criteria). Cross-site inter-rater reliability for diagnostic measures was monitored throughout the study (Deshpande et al., 1998). All cases from the U.S. sample were Caucasian, and when available both parents of the probands were recruited (U.S., n = 151 families; India, n = 141 families). Anonymized cord blood samples from Caucasian live births at a local Pittsburgh hospital served as unscreened, community based controls in the U.S. sample (n = 274). These samples are completely independent of those previously reported by our groups (Nimgaonkar, Zhang, Caldwell, Ganguli, & Chakravarti, 1993), (Nimgaonkar et al., 1996), (S. Prasad et al., 1999).

The study was approved by Institutional Review Boards (IRBs) at the University of Pittsburgh and Dr. Ram Manohar Lohia Hospital, New Delhi. Written informed consent was obtained from all participants, but was not required for the anonymous neonate DNA according to the University of Pittsburgh IRB regulations.

3.3.2 Laboratory

Venous blood was obtained from all participants and genomic DNA extracted using the phenol chloroform method. Using pooled DNA samples from Caucasian SZ/SZA cases (n = 100), we sequenced 500 - 700 bp amplicons extending over all *DRD3* exons and exon-intron boundaries. We also sequenced amplicons spanning reported database SNPs that were available at the time (http://www.ncbi.nlm.nih.gov, NT_005795.5, Hs3_5952, chromosome 3 working draft sequence; 2001). In total, 40 amplicons were surveyed

spanning 109 kb genomic region within and flanking *DRD3*. Sequencing was performed using Big Dye Terminator kit v3.1 (Applied Biosystems, Inc.) and isopropanol precipitation followed by centrifugation. Using this method, we were able to identify SNPs with minor allele frequencies over 5-10% (Chowdari et al., in review). A total of 20 common SNPs were identified from the 40 amplicons (only rs6280 and a rare SNP in LD with rs6280, rs3732783, were detected from the exon screening). Following SNP identification, amplicons spanning polymorphisms were re-sequenced in a panel of 24 unrelated Caucasian individuals. Linkage disequilibirum (LD) analysis was then evaluated in these individuals.

3.3.3 SNP Selection

We found that 9 "tag" SNPs were required to reasonably represent all 20 SNPs identified, i.e. the pair-wise correlation of genotypes as assessed by r^2 was greater than 0.8 between loci and minimal information was gained by analyzing all SNPs independently. Two other SNPs (rs1503670, rs1800828) had been investigated in previous studies and were added for replication purposes (Anney et al., 2002) (Ishiguro, Ohtsuki et al., 1998) (Sivagnanasundaram et al., 2000) (Staddon et al., 2005). Two redundant SNPs within intron 1 were also included. 13 total SNPs were therefore genotyped in the U.S. sample (see Figure 3; Table 3).

In the Indian sample, we evaluated 141 case-parent trios. Analyses were initiated with all 13 SNPs from the U.S. panel, but two were discarded due to failed genotyping assays (rs7625282 and rs7616367). Of these SNPs, one was a redundant SNP and the information loss was minimal (rs7625282). The second (rs7616367) was only in modest

LD ($r^2 > 0.5$) with a successfully assayed SNP (rs2399504) and some information loss resulted.

3.3.4 Genotyping

A PCR based assay using a single base extension method was used to gentoype all samples (SNaPshot; ABI Biosystems Inc.). Interplate and intraplate duplicate samples, as well as water negative controls, were used as quality control measures in all assays. In addition, one SNP (rs2134655) was typed in duplicate on 384 samples to estimate error rates. Two individuals read genotypes independently, blind to clinical status. In case of ambiguous calls, samples were re-assayed or sequenced.

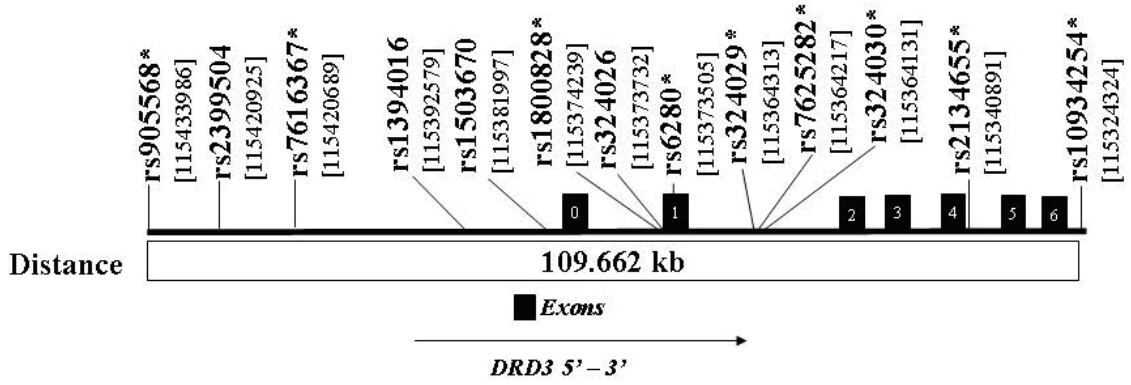

Figure 3 DRD3 genomic organization and variants studied in US sample

*Dopamine D3 receptor (DRD3) genomic organization and SNPs investigated in the U.S. sample. Known exons are numbered, as well as an additional exon (numbered 0) suggested by Anney et al. (Anney et al., 2002). Chromosome location of each SNP based on dbSNP build 128 provided in brackets. *Denotes associated SNP in U.S. analyses.*

3.3.5 Statistical Analysis

We tested for Mendelian inconsistencies (O'Connell & Weeks, 1998) in the family samples and Hardy-Weinberg equilibrium in the case-control samples (GENEPOP software, version 1.31). Differences in genotype distributions between cases and controls for individual SNPs were assessed using the Armitage Trends test (SAS software). Haplotype frequencies were estimated with PHASE software (version 2.0.2) (J. C. Stephens et al., 2001) (M. Stephens & Donnelly, 2003). We tested for haplotype associations using SNPEM software (D. Fallin et al., 2001). Where significant, global haplotype results were retested with COCAPHASE from the UNPHASED software suite (version 2.403; http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/). COCAPHASE uses a method of standard unconditional logistic regression. Correction for multiple tests was performed using 5,000 permutations. For these analyses, "case" and "control" status are reassigned, and in each replicate all the selected markers are analyzed and the most significant p-value stored. As a result, the permutation procedure gives a significance level corrected for the multiple haplotypes and markers tested. Permutation test results from COCAPHASE are reported for global analyses.

Family-based associations were evaluated for individual SNPs / haplotypes using the transmission disequilibrium test (TDT; (Kruglyak, Daly, Reeve-Daly, & Lander, 1996)). Global tests assessing transmission distortion were performed using TRANSMIT software (Clayton, 1999), and significant results were retested with the permutation test available through FBAT software (100,000 permutations) (http://www.biostat.harvard.edu/~fbat/fbat.htm). Good agreement in p-values was found between tests, and FBAT results are reported here.

Linkage disequilibrium (LD) was evaluated using a clustering algorithm available in Hclust software (Rinaldo et al., 2005). Briefly, Hclust computes a similarlity matrix from the square of Pearson's correlation (r^2) between allele counts at pairs of loci, then uses hierarchical clustering to group correlated SNPs. For some analyses, we were interested in identifying a subset of SNPs that are reasonably correlated with all other SNPs in the dataset, i.e. "tag" SNPs. Hclust identifies a set of tag SNPs based on the user specified minimum correlation between SNPs within a cluster. We used a conservative cutoff in which allele counts between all SNPs in a cluster had a correlation of $r^2 > 0.8$.

3.3.5.1 Genomic Control

To control for possible population sub-structure in our case-control sample, we employed a variation of the original genomic control method described by Devlin and Roeder (Devlin & Roeder, 1999) (Bacanu et al., 2000) (Devlin, Roeder, & Bacanu, 2001). We assessed a pool of 420 SNPs from 63 independent genomic regions that had been previously chosen for schizophrenia association studies and genotyped in these samples. Since these SNPs were selected on the basis of being either functional or positional schizophrenia candidates, these analyses were biased towards an a priori hypothesis of association and may represent a more conservative correction than proposed originally by Devlin and Roeder. From this pool, we randomly selected one SNP per region (63 SNPs, total) and determined the median chi square test statistic for case-control comparisons across these SNPs. This process was repeated 10,000 times to yield a distribution of median chi square test statistics (df = 1). We then divided the mean of this result by the expected median of the chi square distribution with one degree of freedom (0.456) to

generate a single correction factor for all of our SNP based case-control analyses previously described (Bacanu et al. 2000).

3.4 RESULTS

3.4.1 Quality Control

Sequencing was performed for all SNPs using DNA from 24 unrelated parents from among the Caucasian U.S. case-parent trios. Sequencing traces were compared with genotype calls from the SNaPshot assays and no inconsistencies were found. We genotyped one SNP, rs2134655, in duplicate for 384 samples and found no discrepancies. Four Caucasian cord samples were also used as positive controls in all genotyping from the U.S. and Indian samples (4 per 96-well plate, 56 total duplicate genotypes), and no discrepancies were detected. Genotype failure rates were low for all samples, and rates were similar between populations studied (U.S., mean failure rate per SNP = 0.0172, standard deviation = .0157, range 0.0 - 0.067; India, mean = 0.0424, standard deviation = 0.0156, range 0.018 - .070). We performed checks for Mendelian inheritance inconsistencies and found no Mendelian errors in the U.S. sample (13 SNPs, 151 trios) or the Indian sample (11 SNPs, 141 trios). We tested for deviations from HWE among all sample groups (controls, parents, cases) for all SNPs. The U.S. cases deviated from HWE at rs6280 ($p = 0.025$). We found no deviations from HWE in the Caucasian cords or parents. In the Indian sample, we found deviation from HWE in the parents for one SNP ($p = 0.035$), rs905568.

3.4.2 Comparison of linkage disequilibrium (LD) between samples

We performed LD analyses for the eleven SNPs genotyped in both samples using the community-based controls and parents of probands in the U.S. sample (n = 576), and the parents in the Indian sample (n = 282). Our results suggested similar patterns of LD, SNP clusters, and tag SNPs between the two samples (Figure 4).

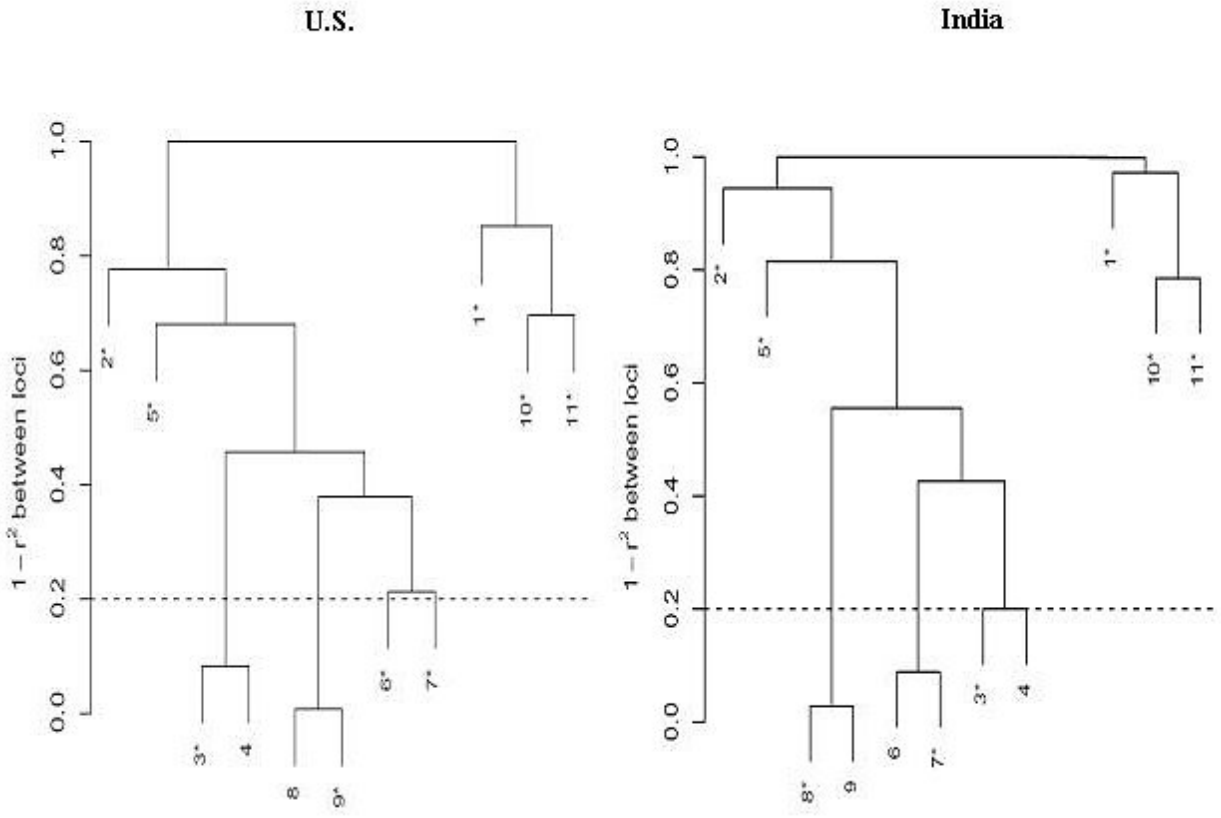

Figure 4 Linkage disequilibrium between SNPs analyzed in both US and Indian samples

Cluster dendrogram for SNPs genotyped in both samples shows similar patterns of LD. "Tag" SNPs are denoted by an asterisk (*), and the tag SNP set is identical between samples. Analysis restricted to the 11 SNPs genotyped in both samples. SNP numbers correspond to Table 3.

3.4.3 Association testing in U.S. sample

3.4.3.1 SNP analyses

In the U.S. sample, significant differences in genotype distributions between cases and controls were detected for three SNPs (rs905568, $p < 0.001$; rs6280, Ser allele, $p = 0.001$; rs2134655, $p = 0.022$; see Table 1). A trend for association was detected at rs10934254 ($p = 0.073$). We did not find a significant increase in homozygosity among cases at rs6280 (ser/ser, ser/gly, gly/gly genotype counts: cases 173 / 136 / 12, controls 119 / 127 / 26, respectively). Of these SNPs, only rs6280 has been investigated in previous studies.

Our genomic control analyses of the median χ^2 distributions from SNPs within 63 independent genomic regions sampled 10,000 times yielded a mean of 0.57. When dividing this by 0.456, the expected median of a χ^2 distribution with 1 d.f. (see Bacanu et al. 2000), we derived a correction factor of 1.25. All SNP based analyses were re-analyzed using this correction, and the results are displayed in Table 3.

Family-based analyses detected significant transmission distortion at six SNPs ($p < 0.05$; see Table 1), including four SNPs confined to the region from intron 1 to 5.9 kb downstream of *DRD3* (rs324029, rs7625282, rs324030, rs10934254; see Table 1). Alleles at rs6280 were not significantly over-transmitted in these analyses ($p = 0.13$).

Table 3 *DRD3* SNP-based results across samples

						U.S. Samples					India Trios	
#	SNP	Gene Region	Nuc	Strand	Allele Code	[a]Freq. *(Case/ Control)*	[b]Case-Control p-value	[c]GC p-value	[d]TDT (T/NT)	TDT p-value	TDT (T/NT)	TDT p-value
1	rs905568	5'	C	+	2	.48/.37	*<0.0001*	0.0008	78/72	0.62	51/47	0.69
2	rs2399504	5'	C	+	1	.82/.81	0.923	0.933	49/36	0.16	28/27	0.89
--	rs7616367	5'	A	+	2	.74/.74	0.944	0.95	66/43	*0.02*	--	--
3	rs1394016	5'	T*	-	2	.37/.33	0.145	0.194	77/62	0.2	64/46	*0.07*
4	rs1503670	5'	G	+	1	.63/.64	0.72	0.75	83/65	0.14	74/64	0.39
5	rs1800828	5'	G	-	1	.80/.77	0.175	0.23	62/39	*0.02*	59/45	0.17
6	rs324026	5'	T*	+	2	.69/.68	0.62	0.66	78/59	0.1	70/56	0.21
7	rs6280	Exon	A*	-	1	.75/.67	*0.001*	0.004	71/54	0.13	70/60	0.36
8	rs324029	Intron	C*	-	2	.73/.72	0.855	0.87	72/50	*0.04*	60/42	*0.07*
--	rs7625282	Intron	T	-	2	.77/.76	0.776	0.806	71/45	*0.01*	--	--
9	rs324030	Intron	C*	+	2	.72/.73	0.813	0.83	72/49	*0.04*	59/44	0.14
10	rs2134655	Intron	G	-	2	.73/.67	*0.022*	0.075	65/53	0.27	48/42	0.53
11	rs10934254	3'	C	-	1	.44/.39	*0.073*	0.12	80/57	*0.05*	71/48	*0.03*

*Single nucleotide polymorphism (SNP) # is given in sequential order according to DRD3 transcription (5' to 3') from the most upstream (telomeric) to downstream (centromeric) SNP. SNP# is only given for SNPs assayed in both samples. Nuc = nucleotide. Strand = genomic strand genotyped in this study. *Nucleotide provided is designated as "other" allele, not "reference" allele by HapMap (HapMap, 2003). [a]Frequency of the allele provided in cases and controls. [b]Trends test p-values from genotype distributions. [c]GC p-value = p-value after genomic control correction applied. [d]T = transmitted allele, NT = not transmitted allele (transmission disequilibrium test [TDT]).*

3.4.3.2 Haplotype analyses

Haplotype analyses in the case-control sample suggested significant associations with all haplotypes incorporating the significantly associated SNPs. Global tests (SNPEM omnibus likelihiood ratio) supported associations with these haplotypes (data not shown). All results remained significant after permutation testing (COCAPHASE global tests, 1,000 permutations; data not shown). However, to mitigate against false positives in haplotype analyses, we interpreted individual haplotype results as significant only if they were also associated in family-based analyses. Using this criterion, we observed significant over-transmission of all 2, 3, and 4 SNP haplotypes comprised of SNPs from

intron 1 to the 3' region of the gene (see Table 4). Analyses of global transmission distortion across these SNPs supported significant associations (see Table 5). For comparison purposes, these data were re-analyzed for the 11 SNPs available in the Indian sample and results are shown in Table 2.

3.4.4 Replication testing in the Indian family sample

SNP-based analyses of 11 SNPs detected a significant association with rs10934254 in the *DRD3* downstream region (Table 1). Significantly increased transmission was observed with the same allele as the U.S. sample (rs10934254, transmitted alleles / untransmitted alleles: U.S., 80/57; Inidia, 71/48). Non-significant over-transmission of the same alleles significantly associated with schizophrenia in the U.S. sample was observed for two additional SNPs within intron 1. As in the initially tested U.S. sample, we found significant over-transmission of a common haplotype spanning intron 1 to the 3' region of *DRD3* (markers rs324029-rs324030-rs2134655-rs10934254). The associated alleles comprising this common haplotype in the Indian sample were identical to those in the U.S. sample (see Table 4). Global tests of transmission distortion at these SNPs also supported associations in this cohort (see Table 5).

We conducted exploratory analyses in an attempt to explain inconclusive results from previous studies. We investigated whether associations at rs6280 could be attributed to differing haplotype backgrounds. To accomplish this, we separately combined rs6280 with haplotypes spanning the 5' region of the gene and haplotypes 3' to exon 1. We first

explored associations in our U.S. case-control sample, then our U.S. family sample. If results were consistent, we tested for replication in the Indian cohort.

Similar to inconsistent replications in previous studies, we detected case-control differences at rs6280 in the U.S. sample, but neither family sample (Table 1). Significant associations were also not detected with either allele when combined with other SNPs 5' to the gene, as was done in previous studies (Anney et al., 2002) (Ishiguro, Ohtsuki et al., 1998) (Sivagnanasundaram et al., 2000) (Staddon et al., 2005). However, when rs6280 was combined with the associated common haplotype in both samples from our initial analyses (SNPs 3' to exon 1), significant associations were consistently observed with the Ser allele (Table 2). These results were replicated in the Indian sample for the same common haplotype, and global tests incorporating these SNPs were significant across study designs in both samples (see Table 2). Associations were not detected with the Ser or Gly alleles against any other haplotype backgrounds (see Table 3 for all haplotype frequencies). The allele encoding glycine (G) is rarely carried against this haplotype background (frequency of Gly-C-G-G-C = .003; Table 3), and the Ser allele was actually *under*-represented in cases as compared to controls against other haplotype backgrounds in the U.S. sample. Taken together, these results could account inconsistencies in studies assessing only rs6280.

Table 4 Haplotype results incorporating associated intronic and downstream SNPs

		U.S. Samples						*Indian Samples*		
		Case-Control			*Families*			*Families*		
[a]SNPs in haplotype	[b]Alleles	*[c]Freqs (Case / Control)*	*Hap [d]p-value*	*Global [e]p-value*	*T/NT*	*TDT [f]p-value*	*Global [g]p-value*	*T/NT*	*TDT p-value*	*Global p-value*
8-9	2-2	0.72/0.72	0.81	0.81	58/34	0.01	0.03	46/28	0.04	0.08
9-10	2-2	0.47/0.39	0.03	0.04	69/32	0.0002	0.0002	45/38	0.44	0.34
10-11	2-1	0.50/0.42	0.01	0.05	60/33	0.005	0.04	57/31	0.005	0.002
8-9-10	2-2-2	0.46/0.38	0.02	0.01	68/31	0.0002	0.0001	44/35	0.31	0.41
9-10-11	2-2-1	0.39/0.34	0.039	0.002	53/26	0.002	0.009	35/19	0.03	0.006
8-9-10-11	***2-2-2-1***	***0.39/0.34***	***0.039***	***0.002***	***52/26***	***0.003***	***0.009***	***36/17***	***0.009***	***0.005***
7-8-9-10-11	***1-2-2-2-1***	***0.39/0.32***	***0.01***	***0.002***	***50/24***	***0.002***	***0.02***	***31/14***	***0.01***	***0.03***

[a]SNP numbers correspond to numbers provided in Table 1. [b]Alleles given correspond to reference allele # given in Table 1. [c]Frequency of the over-transmitted haplotype in the U.S. sample for U.S. cases and controls. [d]p-value from individual haplotype case-control comparisons. [e]Global p-value incorporating all haplotypes after correction using 1,000 permutations (Cocaphase). [f]TDT p-value is individual haplotype p-value, and [g]global p-value is whole marker results incorporating all haplotypes after 100,000 permutations (FBAT).

Table 5 Frequency of all common haplotypes spanning exon 1 to the DRD3 3' region

SNPs	Alleles	Case Freq	Control Freq
8-9-10-11	*2221*	*0.39*	*0.34*
	2212	0.26	0.31
	1122	0.22	0.23
	2222	0.07	0.06
	1121	0.05	0.05
7-8-9-10-11	*12221*	*0.39*	*0.32*
	12212	0.26	0.31
	21122	0.17	0.22
	22222	0.03	0.05
	21121	0.04	0.05
	12222	0.04	0.02
	11122	0.05	0.01
	11121	0.01	0
	12211	0	0.01
	22221	*0*	*0*

All common estimated haplotype frequencies (>1%) for SNPs 3' to exon 1 (8-9-10-11) alone and when combined with rs6280 (7-8-9-10-11). The associated haplotype is given in bold and italicized. Alleles correspond to the reference allele number given in Table 1. The Ser allele at rs6280 (SNP 7) corresponds to allele 1, Gly is allele 2.

3.4.5 Survey of publicly available variations at *DRD3*

As these SNPs were identified and genotyped in the initial U.S. sample prior to the first release of the International Hapmap Project (IHP) (HapMap, 2003), additional information has become available regarding other SNPs in the region. Thus, we conducted post-hoc LD analyses to evaluate the coverage of this region in the current and previous studies. We genotyped the 13 SNPs from the current study in 90 CEPH (Utah residents with ancestry from northern and western Europe) individuals and included available SNPs from IHP as of June, 2005. When combined, data was obtained for 50 SNPs across the 109 kb, 35 of which had an MAF > 5%. Our analyses indicated 16 tag SNPs would reasonably represent all available SNPs with MAF > 5% (r^2 > 0.8), 8 of

which were genotyped in the current study (50%), and 22 SNPs would be required to tag all variations with MAF greater than 1% (42 total SNPs). We also find that rs6280 was highly correlated with 5 SNPs 5' to the gene, but did not tag any available SNPs 3' to exon 1. These results suggest previous studies assessing only rs6280, or SNPs 5' to exon 1, have not adequately accounted for variants 3' to exon.

To determine whether our haplotype results could be a consequence of the observed association with rs6280, we estimated LD (r^2) between the associated haplotype spanning SNPs rs324029 – rs324030 – rs2134655 – rs10934254 and rs6280 in the unrelated US controls (n = 278). We find that rs6280 is not in substantial LD with the associated common haplotype ($r^2 < 0.212$), suggesting some degree of independence between the associated haplotype and rs6280.

3.5 DISCUSSION

Recent simulations have suggested that in the presence of an unidentified liability locus, patterns of associations can be complex, depending on the test statistics used. Indeed, the liability locus may not produce the maximum test statistic (Roeder et al., 2005), which is instead found at SNPs in significant or even modest linkage disequilibrium (LD) with the liability locus. It is often beneficial to therefore analyze a large set of polymorphisms for candidate gene association studies. The rapid identification of polymorphisms in the human genome, their availability in the public domain, and accompanying LD analyses have enabled such studies. Thus, it is feasible and potentially necessary to revisit earlier association studies that typically investigated

only one polymorphism at a gene, such as *DRD3*. The initial report by Crocq and colleagues in two samples suggested associations between schizophrenia and rs6280 with a substantial effect size (pooled relative risk in homozygotes = 2.61) (Crocq et al., 1992). Replication was pursued in an enormous number of subsequent studies with inconsistent results, and the largest in a series of meta-analyses (Jonsson, Flyckt et al., 2003) has suggested this association may not be present across populations. However, since most studies investigated only this SNP, it is uncertain whether there are other, more relevant associations at this gene.

Using rigorous quality control measures, we tested associations at *DRD3* with two different study designs (case-control and family based) and two independent samples. These samples have not been investigated in prior reports on *DRD3* by our groups (Nimgaonkar et al., 1993), (Nimgaonkar et al., 1996), (S. Prasad et al., 1999). Our primary goal was to seek consistency in associations across differing study designs and populations. We also sought explanations for the inconsistencies reported for rs6280.

Our initial comparisons in the U.S. Caucasian cases and community based controls revealed significant associations for three SNPs and related haplotypes. We did not detect increased rates of homozygosity among the cases at rs6280. Since the associations could be confounded by unknown population substructure, we re-evaluated our results using a potentially conservative variation of genomic control. The results from two SNPs remained significant, despite this conservative correction.

We next evaluated approximately half of the U.S. cases for transmission distortion using the TDT. Even in this smaller sample, we detected associations at four SNPs. However, there was no overlap with the associated SNPs from the case-control

comparisons. Inconsistencies in the pattern of associations with different SNPs between case-control and family based analyses, similar to those observed here, can result from differing assumptions, power, and properties of these tests (Bacanu et al., 2000). Supporting our initial results, we detected association with one of the SNPs that displayed transmission distortion in the U.S. families (rs10934254) in a second Indian cohort. Consistent with the similar patterns of LD observed between variations from the Indian and U.S. populations, the same common haplotype was over-transmitted in both samples. This haplotype was also over-represented in the entire sample of U.S. cases when compared with the U.S. community-based controls.

Taken together, our analyses indicate an association with a common haplotype in both samples. The results are intriguing as they were replicated through three different sets of analyses and two independent populations. While it is possible that this haplotype itself conveys liability to schizophrenia, given the relatively sparse SNP density evaluated in this region, we speculate it is more likely that our analyses are suggestive of a risk allele(s) carried against this common background. As our most significant haplotype result in the U.S. sample spanned rs324029 – rs2134655, these findings may implicate a polymorphism generating schizophrenia susceptibility in the region spanning intron 1 to the exon / intron boundary 3' to exon 4 at *DRD3*.

If true, these findings yield a plausible explanation for the inconsistencies observed in the past regarding the association between rs6280 and schizophrenia. We observed increased transmission of the Ser allele against the associated haplotype background in both the U.S. and Indian samples, but not other haplotypes. Our analyses suggest rs6280 is in modest LD with the common haplotype associated with

schizophrenia in the current study. Consistent with results of others regarding association tests (Roeder et al., 2005), it is possible that rs6280 could produce the maximum test statistic in some, but not all, previous studies due to these LD patterns. Our analyses bear this out, as rs6280 was significantly associated in our U.S. case-control analyses, but not our family based analyses.

Though consistent, our results cannot be considered conclusive for several reasons. First, although 1,332 persons have been analyzed, the sizes of the individual samples are still modest. Second, as in most association studies, a relatively large number of individual tests were conducted. Although some SNPs are correlated and tests are not completely independent, the results presented here are not corrected for multiple comparisons. On the other hand, we interpreted haplotype results as significant only if they were supported in both U.S. study designs, and replicated in the Indian sample. The associations detected remained significant after permutation testing, global analyses were conducted for haplotypes showing significant associations using two different types of analytical software, and a potentially conservative genomic control correction was used to evaluate the case-control SNP results. Nonetheless, further replication is necessary to confirm these findings.

A third concern is the absence of a clear functional basis for the observed associations. $Ser^{9}Gly$ remains the only known polymorphism that alters the protein structure at this gene. Previous studies seeking exonic mutations have found synonymous changes at exon 1 (Griffon et al., 1996) and exon 3 (Asherson et al., 1996), but these SNPs are uncommon. Ashershon and colleagues also found an intronic 5 bp deletion polymorphism flanking exon 5 of the gene with similar frequency among cases

and controls (7-8%) (Asherson et al., 1996). These results may warrant further investigation given our current findings of association with a common haplotype spanning this region. While it is possible that these SNPs, or SNPs against this common background, have a functional effect at this gene, at present there is no clear molecular explanation for these results. The lack of a functional implication is an important hurdle and plagues many well accepted genetic associations; e.g., *RET* gene and Hirschsprung's disease (Emison et al., 2005).

In conclusion, we report novel, replicable associations with schizophrenia at the D3 dopamine receptor (*DRD3*). Our results indicate that serine allele of $Ser^{9}Gly$ may not be the only susceptibility allele at this gene. Complete polymorphism screening to identify all human variation across this region, comprehensive LD mapping, evaluation of conserved regions across species, and analyses in a sufficiently powered cohort are necessary in order to provide more convincing evidence for this locus as a susceptibility factor in schizophrenia pathogenesis. These efforts are currently ongoing.

3.6 ACKNOWLEDGMENTS

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4.0 STUDY #2: A NETWORK OF DOPAMINERGIC GENE VARIATIONS IMPLICATED AS RISK FACTORS FOR SCHIZOPHRENIA

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4.1 ABSTRACT

We evaluated the hypothesis that dopaminergic polymorphisms are risk factors for schizophrenia.

Stage I (screening): Eighteen dopamine-related genes were analyzed in two independent US Caucasian samples: 150 trios and 328 cases / 501 controls. The most promising associations were detected with *SLC6A3* (alias DAT), *DRD3*, *COMT*, and *SLC18A2* (alias VMAT2).

Stage II (SNP coverage and epistasis): To comprehensively evaluate these four genes, 68 SNPs were genotyped in all 478 cases and 501 controls from stage I. Fifteen (23.1%) significant associations were found ($p \leq 0.05$). We tested for epistasis between pairs of SNPs providing main effects and observed 17 significant interactions (169 tests); 41.2% of significant interactions involved rs3756450 (5' near promoter) or rs464049 (intron 4) at SLC6A3.

Stage III (confirmation): Sixty-five SNPs were genotyped in 659 Bulgarian trios. Both *SLC6A3* variants implicated in the US interactions were over-transmitted in this cohort (rs3756450, $p = 0.035$; rs464049, $p = 0.011$). Joint analyses from stages II and III identified associations at all four genes ($p_{joint} < 0.05$). We tested 29 putative interactions from stage II and detected replication between 7 locus pairs ($p < 0.05$). Simulations suggested our stage II and stage III interaction results were unlikely to have occurred by chance ($p = 0.008$ and 0.001, respectively).

Stage IV (function): We tested rs464049 and rs3756450 for functional effects and found significant allele specific differences at rs3756450 using EMSA and dual-luciferase promoter assays.

Conclusions: Our data suggest a network of dopaminergic polymorphisms increase risk for schizophrenia.

4.2 INTRODUCTION

The distribution of schizophrenia in families and populations is consistent with a substantial genetic basis for the disorder. No obvious genetic model can explain the data, but models including multiple interacting loci conferring risk provide a good fit (Risch, 1990; Schliekelman & Slatkin, 2002). The disorder is common, with an estimated lifetime morbid risk of 1%, and concordance estimates for monozygotic twins (48%) is significantly higher than that for dizygotic twins (17%) (I. Gottesman, 1991). There has been long-standing research into the hypothesis that dopamine dysfunction contributes to schizophrenia pathogenesis (Laruelle et al., 1999; D. A. Lewis & Lieberman, 2000). The hypothesis originated from observed correlations between the clinical potency of anti-psychotic drugs and their affinity for dopamine D2 receptors (DRD2) (Carlsson & Lindqvist, 1963; Creese et al., 1976; Seeman et al., 1976). Patients with schizophrenia display increased sensitivity to the psychotogenic effects of agents that increase synaptic dopamine release (Angrist & van Kammen, 1984; Davidson et al., 1987; Laruelle & Abi-Dargham, 1999; Laruelle et al., 1999; Lieberman et al., 1984). In addition, acute amphetamine challenge to schizophrenia patients leads to increased dopaminergic

transmission in vivo, as measured by radioligand binding to dopamine D2 receptors during positron emission tomography (PET) scans (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). Therefore, dopamine genes have traditionally been prime candidates for genetic studies in schizophrenia.

Despite the substantial biological evidence implicating dopaminergic dysfunction in schizophrenia pathogenesis, it is not precisely known whether genetic polymorphisms in dopaminergic genes are associated with dopamine abnormalities. If such a functional link exists, the nature of these variations, the number of genes affected, interactions amongst them, and their functional importance is poorly understood. Associations between schizophrenia and many dopaminergic gene variations have been reported, but most studies evaluated one or at best a handful of polymorphisms, usually based on preliminary evidence of a functional impact (e.g. exonic SNPs or functional repeats). Most previous studies were better suited to identify risk factors of substantial effect size than multiple interacting loci, for which the marginal effect of an individual locus could be small. Therefore, it appears that many genes in the dopamine pathway have not been investigated adequately for their impact on schizophrenia risk. Our recent review of the literature estimated that roughly 5% of representative common SNPs currently available in public databases have been considered in association studies of dopaminergic genes with at least 50% power to detect modest effect sizes expected (odds ratios from 1.2 – 1.5) (Talkowski, Bamne, Mansour, & Nimgaonkar, 2007). For example, a large number of studies investigated a single coding variant (rs6280) at the dopamine D3 receptor gene (DRD3) with largely inconsistent results (Ioannidis et al., 2001; Jonsson, Flyckt et al., 2003; Jonsson et al., 2004). Until recently, studies did not consider other variations

within the gene. Two independent studies of 13 SNPs and 17 SNPs now suggest associations with other SNPs / haplotypes might account for past inconsistencies at rs6280 (Dominguez et al., 2007; Talkowski, Mansour et al., 2006). Similar associations could be present with common variants yet to be investigated at other dopaminergic targets, but alternative strategies may be necessary to jointly evaluate these genes.

Multi-stage studies can be useful in analyses of a functionally related network of genes by initially screening a large group of susceptibility targets and subsequently evaluating only the most promising candidates in additional samples, thus maximizing power with the resources available (Aplenc, Zhao, Rebbeck, & Propert, 2003; Lowe et al., 2004; Satagopan & Elston, 2003). Skol et al. recently showed that an increase in power for multi-stage whole genome studies can be attained by evaluating the joint distribution of test statistics from both samples versus independent consideration of each sample (Skol et al., 2006). We reasoned a similar approach could be applied to gene-based association studies that are restricted to a smaller number of loci, since samples from individual studies are almost always underpowered to consistently detect associations and interactions of modest effect. In the present study, we revisited the genetic basis for the so called 'dopamine hypothesis' of schizophrenia by investigating eighteen dopaminergic genes in three independent samples. We hypothesized that key susceptibility variants within the dopaminergic network could be identified if results from multiple samples were evaluated jointly. Our multi-stage strategy progressively pruned the list of promising susceptibility candidate genes and culminated in functional analyses of associated SNPs.

4.3 METHODS

4.3.1 Samples

Unrelated patients from the US were recruited at Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania and surrounding regions (n = 478). Diagnoses were based on the Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994), supplemented by medical records and informant interviews. Consensus DSM IV diagnoses of schizophrenia (schizophrenia; n = 272) or schizoaffective disorder (schizophreniaA; n = 206) were assigned by board-certified psychiatrists / psychologists following review of all these sources of information. Both parents of 150 patients were ascertained for family based analyses (150 trios). Control DNA samples were collected from the cord blood of 501 unscreened Caucasian neonates born at Magee-Women's Hospital, Pittsburgh, PA. Ancestry and gender was available for all samples.

The Bulgarian patients and their parents were recruited in Bulgaria as part of a collection of parent – proband trios described previously (Kirov et al., 2004). Diagnoses were made according to DSM-IV criteria, following assessment by a psychiatrist using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN, Wing et al 1990) which has been validated for use in the Bulgarian language by one of the authors of the SCAN instrument, and inspection of hospital discharge summaries. In cases where the information collected did not allow a confident diagnosis, the patient was re-interviewed by Dr. Kirov or the clinical coordinator of the project. All patients and their parents received written information on the project and signed an informed consent form. The

Bulgarian sample included 659 trios (total n = 1,977). Probands were diagnosed with schizophrenia (n = 576) or schizophreniaA (n = 83).

The University of Pittsburgh Institutional Review Board approved the study. Written informed consent was obtained from all participants, except neonatal controls, in accordance with IRB guidelines. Ethics Committee approval was obtained from Ethics Committees in all regions of Bulgaria where families were recruited.

4.3.2 Polymorphism Selection

We initially selected SNPs from the Celera database (Celera, 2003), the most comprehensive source available when stage I analyses were initiated. SNPs were selected based on physical distance (1 SNP / 5 kb attempted). A denser set of SNPs were then chosen for four genes: *SLC6A3*, *SLC18A2*, *DRD3*, and *COMT* in stage II. Polymorphisms for each gene were obtained by surveying the genomic region spanning the gene and at least 5 kb of flanking sequence. For *SLC6A3* and *SLC18A2* SNPs were identified from available HapMap data (HapMap phase I, October 2005 release for *SLC6A3* and HapMap phase II, June 2006 release for *SLC18A2*) (HapMap, 2003) and tag SNPs were chosen using Hclust software (Rinaldo et al., 2005). Hclust computes a similarity matrix from the square of Pearson's correlation (r^2) between allele counts at pairs of loci, then uses hierarchical clustering to group correlated SNPs (minor allele frequency > 5%). Tag SNPs were chosen if the correlation between loci was below an arbitrary threshold ($r^2 < 0.8$). One redundant SNP (rs456082) was also chosen. At *COMT* and *DRD3*, additional SNPs were obtained from in-house sequencing. For *DRD3*, we sequenced overlapping 600 – 800 bp amplicons across the entire gene and 5 kb of

flanking sequence in a pool of 200 Caucasian cases to detect common polymorphisms (minor allele frequency > 5%). When polymorphisms were detected that were not available in HapMap, we sequenced the same 60 unrelated CEPH individuals used by HapMap to investigate patterns of LD. Sixty-nine polymorphisms were detected, 15 of which were novel, and 18 SNPs were selected (see Supplementary Table 4.1). The 18 SNPs included tag SNPs (chosen with Hclust as above) and SNPs associated with schizophrenia in our previous study (Talkowski, Mansour et al., 2006). At *COMT*, 27 SNPs were identified from directly sequencing coding regions and flanking intronic sequence for exons 2-6 as well as the proximal promoter region for S-COMT within intron 3. Sequencing was performed among 60 Caucasian US subjects (data provided by R. Weinshilboum, M.D. and J. Zhang, Ph.D., Mayo Clinic, Rochester, MN; see (Shield, Thomae, Eckloff, Wieben, & Weinshilboum, 2004) for details). Individuals used for these analyses were different than those used in HapMap. In sum, we chose 20 SNPs from the combination of HapMap and individual sequencing, realizing redundancy in SNP selection could result. It should be noted that for *COMT*, some SNPs obtained within the pre-determined flanking sequence were localized to other genes (*ARVCF* or *TXNRD2*), however for clarity these SNPs are still referred to as "COMT" SNPs. In the Bulgarian sample, 65 SNPs were genotyped. Where possible, identical SNPs to stage II were analyzed (n = 59 SNPs). Four additional SNPs were genotyped as surrogates for stage II tag SNPs, and two functional synonymous SNPs at *COMT* were genotyped based on work described by Nackley and colleagues during the course of this study (Nackley et al., 2006) (Supplementary Table 2).

Since a case-control design was used in stage II, we sought evidence for population substructure by implementing genomic control (GC) analyses using 31 SNPs (Bacanu et al., 2000; Devlin & Roeder, 1999). We chose a single common SNP (minor allele > 10%) from each of the 31 genomic bins least likely to be linked to schizophrenia from a meta-analysis of linkage scans by Lewis and colleagues (C. M. Lewis et al., 2003).

4.3.3 Genotyping Assays

4.3.3.1 Stage I

The screening SNPs (n = 95) were assayed using multiplexed polymerase chain reaction (PCR) amplification followed by single base extension (SNaPshot, ABI Biosystems), as described elsewhere (Mansour et al., 2005).

4.3.3.2 Stage II

SNPs were genotyped using the hybridization based Illumina Golden Gate assay, as described elsewhere (Shen et al., 2005). In sum, 99 SNPs were assayed, including 31 GC SNPs. The median trends test statistic for genomic control was 0.336 (expected median = 0.456), yielding no evidence for noteworthy sub-structure. Hence corrections were not applied as it would be anti-conservative (Devlin & Roeder, 1999).

4.3.3.3 Stage III

Genotyping in the Bulgarian sample was conducted at both Cardiff University (Cardiff, Wales, UK) and the University of Pittsburgh (Pittsburgh, PA, US). At Cardiff, 33 of the

SLC6A3 and *DRD3* SNPs were genotyped by the Sequenom MassARRAY™ system using iPlexTM chemistries according to the recommendations of the manufacturers (Sequenom, San Diego, California, USA, http://www.sequenom.com). At Pittsburgh, SNPlex (Tobler et al., 2005) and SNaPshot assays (ABI Biosystems Inc) were utilized to type the remaining 32 SNPs.

4.3.4 Quality control

In the stage I family based analyses, we sequenced 8 cases for all SNPs (752 sequenced genotypes) and one discrepancy was observed between the sequencing data and the SNaPshot data. In stage I and II case-control analyses, there was complete concordance between Illumina genotypes and HapMap genotypes for 11 CEPH individuals. Among 3,024 duplicated genotypes from positive controls, no discrepancies were found. The overall genotype call rate for stage II was 99.83%. In stage III, we assayed 31 CEPH individuals (n = 2139 genotypes) and found 5 discrepancies. In addition, four SNPs (rs464049, rs463379, rs324030, rs167771) were genotyped in duplicate for all 1977 Bulgarian samples at Pittsburgh and Cardiff (15,816 total genotypes) and 24 discrepancies were found (stage III estimated error rate = 0.0015 - 0.0023). The mean genotype call rate was 95.71%.

Mendelian inconsistencies and deviations from Hardy Weinberg expectations (HWE) for individual SNPs were evaluated using PEDCHECK (O'Connell & Weeks, 1998) and GENEPOP (version 1.31) software, respectively. We detected 9 Mendelian errors among the 95 SNPs assayed in stage I, and 74 Mendelian errors from analyses of 65 SNPs in 659 Bulgarian trios. In sum, 18 families were removed from Bulgarian analyses due to

multiple Mendelian errors, and remaining sporadic errors were set to null. Hardy-Weinberg Equilibrium (HWE) was tested in each population separately (US cases, US controls, US parents, Bulgarian parents, Bulgarian cases.

4.3.5 Electrophoretic Mobility Shift assay (EMSA)

Non-radioactive EMSA for rs3756450 was performed using DIG Gel Shift Kit (Roche Applied Science) according to manufactures protocol with slight modifications. Polyacrylamide gel electrophoresis (PAGE) purified 42-base primers (Integrated DNA Technologies, Inc.) encompassing rs3756450 were annealed to complementary oligonucleotides by incubating them at 95°C for 5 min, followed by gradual cooling to room temperature. Annealed double stranded oligonucleotides were labeled according to the manufacturer's protocol (Roche Applied Science, Inc). Nuclear extracts were prepared from SHSY-5Y cell lines as described (NC Andrews and DV Faller, Nucleic Acids Res. 1991 May 11;19(9):2499). DIG-labeled oligonucleotides were incubated with nuclear extracts (5 μg) in 20 μl reaction containing 5X binding buffer, Poly-L-Lysine, poly[d(I-C)], for 30 min at room temperature. Competitive binding was performed by including 50X unlabelled oligonucleotides in appropriate control reactions. DIG-labeled oligonucleotide-nuclear extract complexes were resolved on 6% non-denaturing polyacrylamide gel for 2 hrs at room temperature and transferred on positively charged nylon membranes (Boehringer Mannheim -Roche Applied Science) by electro-blotting. Blots were visualized by an enzyme chemiluminescent method (Roche Applied Science, Inc). The experiment was replicated, with two fold excess of the nuclear extract added to

reactions having the rs3756450C probes (see Figure 8, lanes 4-6). The primer sequences used for generating allele specific probes are listed with altered bases in red.

rs3756450 T Allele FWD 5' TAGCAGCAACCACAATGATAA**T**AAAGCCGACTTGGCATTTAG 3'
rs3756450 T Allele REV 5' CTAAATGCCAAGTCGGCTTT**A**TTATCATTGTGGTTGCTGCTA 3'
rs3756450 C Allele FWD 5' TAGCAGCAACCACAATGATAA**C**AAAGCCGACTTGGCATTTAG 3'
rs3756450 C Allele REV 5' CTAAATGCCAAGTCGGCTTT**G**TTATCATTGTGGTTGCTGCTA 3'

4.3.6 Dual Luciferase Assay

A 2.8 kb genomic region encompassing the 5' UTR of *SLC6A3* (-2783 to +63, spanning rs3756450) was amplified from two CEPH samples homozygous for alleles of rs3756450, using the Expand High Fidelity PCR System (Roche Applied Science, Inc). The PCR amplified fragments were cloned between Kpn1 and HindIII restriction sites in a pGL3 Basic vector (Promega, Inc). Sequence homology for all residues was confirmed by sequencing. Transient transfections of constructs into neuroblastoma cell line SHSY-5Y (ATCC-CRL-2266) were performed in 24-well plates (0.8×10^6 cells/well) using LipofectAMINE (Life Technologies, Inc.), according to the manufacturer's instructions. The pRL-TK (Promega, Inc) vector expressing Renilla luciferase by a HSV-TK promoter was co-transfected with each construct as an internal control, to normalize for firefly luciferase expression. Cells were harvested 30 h after transfection, and luciferase assays performed using the dual luciferase reporter assay system (Promega, Inc). Relative luciferase values were normalized from a promoter-less pGL3 BASIC vector. Six readings were taken for each clone and the entire experiment was conducted in triplicate.

4.3.7 Statistical Analysis

Evidence for transmission distortion was assessed using FBAT software (Horvath, Xu, & Laird, 2000). Differences in genotype distributions between cases and controls were evaluated with the Armitage Trends test (SAS software) (Devlin & Roeder, 1999). Markers localized to the X chromosome (*MAOA* and *MAOB*) were analyzed using likelihood ratio tests in a loglinear model, as implemented in the UNPHASED software suite (see (Cordell & Clayton, 2002) for review) (Dudbridge, 2003). We tested for gender differences at each of the three *COMT* SNPs previously described by Shifman and colleagues (rs4680, rs737865, rs165599) (Shifman et al., 2002) using logistic regression. Gender comparisons were only made for these three SNPs.

To evaluate results from multiple samples, we computed the joint distribution of test statistics (Z_{joint}), based on the methods of Skol and colleagues (Skol et al., 2006). Here, when combining our results from stages II and III, the proportion of markers genotyped remained the same, and thus Skol et al.'s adjustment for variable number of markers genotyped was not applied. Z-statistics were derived for both case-control and family-based association tests. To calculate Z_{joint}, let n_1 and n_2 be the sample sizes from which test statistics Z_1 and Z_2 were calculated. The formula for Z_{joint} is then:

$$Z_{joint} = SQRT(\pi_1 (z_1)) + SQRT(1-\pi_1 (z_2)), \text{ for which } \pi_1 = n_1 / (n_1 + n_2).$$

It should be noted that the sign of the test statistic (i.e. Z positive or negative) was accounted for in all analyses, meaning the risk allele was required to be the same in both samples. To determine π_1, or the proportion of total samples genotyped in the first stage,

we treated a complete case-parent trio (n = 3 individuals) as the equivalent of one case and one control. For stage I we therefore had the equivalent of 478 cases and 651 controls available, of which the 150 trios represented 26.6% of these samples (i.e. $\pi_{samples}$ = 0.266). In this staged design we calculated Z_{joint} twice, once over the case-control and family-based association analyses in stage I (US samples only), and again over the case-control analyses of stage II and the family-based association analyses of stage III (US and Bulgarian samples). Where surrogates were chosen in the Bulgarian sample to represent tag SNPs in the US sample, Z_{joint} was calculated by combining the test statistic from the original SNP with that of the surrogate. In using this procedure, we are confident that the size of the test was not likely to be altered (e.g. p = 0.05 was still at least a 5% type I error threshold). However, a lower correlation between SNPs could result in loss of power.

Epistatic interactions were tested in stages II and III. Pairs of loci, each of which provided a p-value less than 0.10 for a main effect on risk for schizophrenia, were analyzed for interaction effects using an unconditional logit model for case-control analyses and a conditional logit model for trios (Cordell, Barratt, & Clayton, 2004). In both instances, the "interaction p-value" reported represented the likelihood difference between a full model including both main effects and an interaction term from a reduced model including only main effects. When interaction results were significant by asymptotic approximation, empirical p-values were determined by permutation testing (1,000 permutations; Genetic Association and Interaction Analysis software) (Macgregor & Khan, 2006).

For the functional analyses, we used a paired t-test to determine differences in luciferase activity between the C and T alleles at rs3756450. To determine significant differences between constructs, we conducted analysis of variance.

4.3.7.1 Simulations

Interpretation of the interaction results from the staged design is complicated by the design itself and by the LD structure of SNPs in each gene. To facilitate interpretation of results from interaction tests, we performed a simulation experiment based on the data from these cases and controls. Each simulation consisted of three stages.

Stage (1): permute the case-control status, thus making affection status independent of genotypes while retaining the LD structure of the sample. Test all 68 SNPs individually for association with affection status at two levels of significance ($p \leq 0.05$ and $p \leq 0.10$). If eight or more SNPs are associated at $p \leq 0.05$, then record all *S* SNPs with $p \leq 0.10$ and proceed to Stage 2; else reject this set of data and rerun the permutation until eight or more SNPs are associated at $p \leq 0.05$. Rejection sampling ensures this stage is comparable to the results obtained in the original experiment in terms of the number of SNPs associated with affection status.

Stage (2): using the Stage 1 dataset and the list of *S* SNPs, test for all possible SNP-SNP interactions, with the condition that each of the two SNPs be in different genes (i.e., gene-gene interaction). As per the original experiment, record all *I* interactions having a $p \leq 0.10$ for association.

Stage (3): do a new permutation of case-control data. With these data, test the *I* interactions found in Stage 2, using a significance level of $p \leq 0.05$. Record the number of "replicated interactions" *R*.

We performed this Stage 1 – Stage 3 experiment 10,000 times to obtain the distribution of *R*. This is, in essence, the design of the original experiment. It differs in the sense that the original experiment used a family-based sample in Stage 3 and had slightly different sample sizes, but neither of these features should be important under the null hypothesis evaluated here.

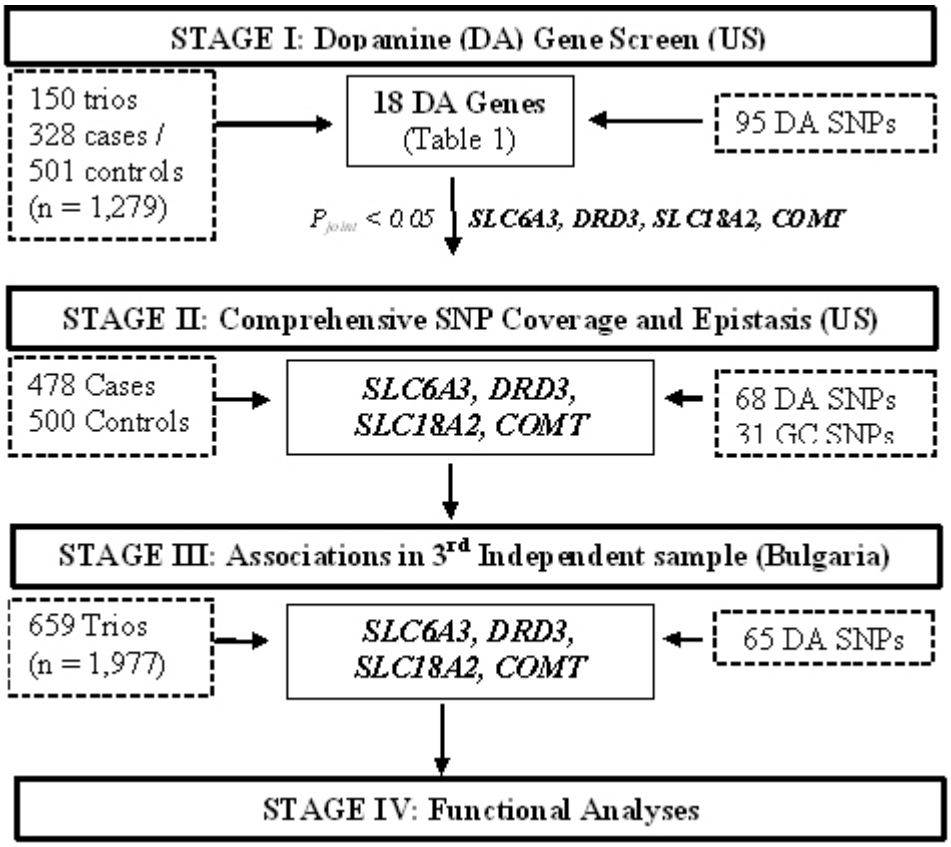

Figure 5 Study Desgin

Overview of multi-stage study design utilized, including all samples and SNPs analyzed in each stage. In stage I, the 328 cases are independent of the 150 probands from the family based samples. dopamine = dopamine, GC = genomic control.

4.4 RESULTS

4.4.1 Design Overview

An overview of the study design is provided in Figure 5. Briefly, in ***stage I*** we screened 18 dopamine-related genes using two independent samples from the US; a family-based sample and a case-control sample. To improve the power of our screen, we evaluated the joint distribution of test statistics from both samples. In ***stage II***, in-depth analyses of the most promising stage I genes were conducted using tag SNPs and all available case-control samples from *stage I*. Pair-wise epistatic interactions were then modeled for a limited number of SNPs where evidence for main effects were detected. In ***stage III***, we analyzed a third independent sample from Bulgaria. In sum, 3,256 participants were genotyped. Finally, functional effects of key SNPs were examined in ***stage IV***.

4.4.2 Candidate Genes

Because the list of genes impacting dopaminergic function is potentially long, subjective, and continually expanding, we restricted our evaluation to dopaminergic genes analyzed in genetic association studies as of 2003. The selected genes included those required for dopamine synthesis (*TH, DDC*), transport (*SLC6A3, SLC6A2, SLC18A1, SLC18A2*), metabolism (*MAOA, MAOB, COMT*), conversion of dopamine to norepinephrine (*DBH*), and all dopamine receptors (*DRD1, DRD2, DRD3, DRD4, DRD5*) (**Table 6**). We also chose three genes important for dopamine regulation, namely *PPP1R1C* (alias dopamineRPP-32), *DRD1IP* (alias CALCYON, a dopamine D1 receptor interacting

protein), and *NR4A2* (alias NURR1, an orphan nuclear receptor and putative transcription factor for the dopamine transporter) (**Table 1**). One candidate, *DRD3*, was analyzed in our U.S. sample earlier as part of a collaborative study (Talkowski, Mansour et al., 2006). Based on the significant associations detected in that study, *DRD3* was retained for stage II of this study, which included 501 independent controls.

Table 6 Dopaminergic genes and SNPs analyzed

				SNPs Genotyped		
Gene	**Location**	**Gene Name (*alias name)***	**Size (kb)**	**Stage I**	**Stage II**	**Stage III**
COMT	***22q11.2***	***catechol-O-methyltransferase***	***27.2***	***7***	**18**	**17**
DBH	9q34	dopamine beta hydroxylase	23	9		
DDC	7p11	dopamine decarboxylase	102.6	5		
DRD1	5q35.1	dopamine D1 receptor	3.1	3		
DRD1IP	10q26.3	D1 receptor interacting protein (CALCYON)	11.5	5		
DRD2	11q23	dopamine D2 receptor	65.6	5		
DRD3	***3q13.3***	***dopamine D3 receptor***	***50.2***	***13***[a]	**18**	**18**
DRD4	11p15.5	dopamine D4 receptor	3.4	3		
DRD5	4p16.1	dopamine D5 receptor	2.1	3		
MAOA	Xp11.3	monoamine oxidase A	90.6	10		
MAOB	Xp11.3	monoamine oxidase B	115.8	6		
NR4A2	2q24.1	orphin nuclear receptor subunit 4 (NURR1)	8.3	5		
PPP1R1B	17q21.2	protein phosphatase 1, regulatory (inhibitory) subunit 1B (dopamineRPP-32)	9.7	4		
SLC18A1	8p21.3	vessicular monoamine transporter, member 1 (VMAT1)	38.4	10		
SLC18A2	***10q25***	***vessicular monoamine transporter, member 2 (VMAT2)***	***35.9***	***3***	**14**	**13**
SLC6A2	16q12.2	monoamine transporter, noradrenaline (NET)	46	8		
SLC6A3	***5p15.3***	**dopamine transporter (DAT, DAT1)**	***52.6***	***6***	**18**	**17**
TH	11p15.5	tyrosine hydroxylase	7.9	3		

Dopamine genes and SNPs analyzed, given in alphabetical order. The bolded genes were further analyzed in Stages II and III. [a]*These SNPs were previously analyzed and results from those published analyses suggested significant associations in these samples (Talkowski, Mansour et al., 2006).*

4.4.3 Stage I: SNP Screen among two independent US samples

We conducted 95 tests of association in the US family based sample (150 trios, SNPs selected from Celera, 2003 based on physical distance). The most significant association was detected at *SLC6A3* (DAT) (rs403636, p = 0.0004, odds ratio = 2.36). Transmission distortion was noted at two other *SLC6A3* SNPs (rs27072, p = 0.0009; rs12516948, p = 0.07). All trends for association (p < 0.10; n = 9 SNPs) were genotyped in a replicate US case-control sample (328 cases, 501 controls). In this independent sample, significant associations were detected with 4 SNPs, including replication of rs403636 (p = 0.04). The joint distribution of test statistics from both samples identified *SLC6A3, DRD3, COMT,* and *SLC18A2* as the four most promising candidates ($p_{joint} < 0.05$) (see Table 7 for selected significant results). These four genes were retained for follow-up analyses.

Table 7 Significant associations from joint analysis of Stage I

				Stage I Families (150 Trios)		Stage I Case-Control (n = 328/501)		Stage I Joint Analysis	
Chr	**Gene**	**SNP**	**BP**	**Z_1**	**P_1**	**Z_2**	**P_2**	**Z_{joint}**	**P_{joint}**
3	DRD3	rs324030	115364131	2.25	0.024	1.48	0.139	2.48	0.013
3	DRD3	rs10934256	115368342	2.62	0.009	1.10	0.271	2.36	0.018
3	DRD3	rs6280	115373505	2.01	0.044	1.70	0.089	2.52	0.012
3	DRD3	rs1800828	115374239	1.97	0.049	1.20	0.230	2.08	0.038
5	SLC6A3	rs27072	1447522	3.26	0.001	1.89	0.059	3.37	0.0007
5	SLC6A3	rs403636	1491354	3.53	4E-04	2.46	0.014	4.00	6E-05
10	SLC18A2	rs3633343	119004938	1.94	0.052	2.09	0.037	2.81	0.005
22	COMT	rs737865*	18310121	1.31	0.190	2.65	0.008	2.93	0.003
22	COMT	rs165815	18334027	1.68	0.090	1.97	0.050	2.55	0.011

*Only SNPs associated based on the joint distribution of test statistics ($p_{joint} < 0.05$) are listed. Z_1, Z_2: Z scores from analysis of family-based and case-control samples, respectively. P_1, P_2: Probability of Z score (p values) from association analyses of family-based and case-control samples, respectively. Z_{joint}, P_{joint}: joint analyses and corresponding p-values when considering test statistics and proportion of total samples genotyped in each design. *analyses in females, conducted based on previous findings by Shifman et al. (Shifman et al., 2002).*

4.4.4 Stage II: Comprehensive coverage and epistasis among US samples

We assayed 68 SNPs among all available cases and controls from stage I (478 cases, 501 controls) at *SLC6A3, DRD3, COMT*, and *SLC18A2*. SNPs were obtained from HapMap (HapMap, 2003) and in-house sequencing for SNP detection. These analyses were not intended to replicate the stage I findings, as the samples overlapped. Instead, they enabled us to conduct in-depth analysis of representative common variants (minor allele > 5%) from these four genes, over and above what was possible in our initial screen.

Overall, the distribution of test statistics from these SNPs was skewed towards small p-values (median trends test = 1.07; expected median = 0.456). Significant associations ($p < 0.05$) were found for 15 SNPs (Supplementary Table 4.1). At *SLC6A3,* 6 of 17 SNPs tested were nominally significant ($p < 0.05$). Linkage disequilibrium analyses (LD) revealed that these associated SNPs were not part of a single cluster (Figure 6). Associations were also detected with six *DRD3* SNPs, three *SLC18A2* SNPs, and one *COMT* SNP.

Gender specific analyses were conducted at three *COMT* SNPs based on a previously reported association by Shifman and colleagues (Shifman et al., 2002). Consistent with those findings, logistic regression revealed a significant interaction between gender and rs737865 genotype ($\chi^2 = 14.14$, 2 d.f., $p = 0.0007$). The significant effect appeared to be attributable to females, and a trends test comparing female patients to female controls for this SNP revealed significant differences in genotype distributions between groups ($p = 0.008$; odds ratio = 1.34). Of note, the frequency of the G allele at

rs737865 among female cases (0.38) was different than all three comparison groups, namely: female controls (0.29), male cases (0.29), and male controls (0.28). Gender related differences were not consistent with Shifman et al. at the other two SNPs (rs165599 and rs4680).

We next tested epistatic interactions among pairs of SNPs from different genes when a main effect was observed (cutoff set at $p < 0.10$, n = 22 SNPs including rs6347 based on stage III, see below; total 169 tests). We identified significant interactions between 17 locus pairs ($p < 0.05$). Notably, 7 of 17 significant interactions (41.2%) involved either rs3756450 in the 5' upstream region of *SLC6A3* or rs464049 within intron 4 of *SLC6A3* (LD between these SNPs: $r^2 = 0.04$ / D' = 0.56). In sum, 29 putative interactions were detected at $p < 0.10$.

4.4.5 Stage III: Corroboration with an independent Bulgarian family sample

Based on our findings in stages I and II, we tested our hypotheses in a third independent sample composed of 659 case-parent trios from Bulgaria (total n = 1,977) using 65 SNPs. Significant associations were again detected in this cohort for both consistently interacting dopamine transporter SNPs in the stage II epistatic analyses (*SLC6A3*: rs464049, p = 0.011 and rs3756450, p = 0.035). Trends for transmission bias ($p < 0.10$) were detected at five *SLC6A3* SNPs. Associations were not detected with other SNPs, including the three key exonic polymorphisms recently shown to alter COMT mRNA secondary structure (rs4680, rs4633, rs4818). We tested rs737865 for gender related differences based on our stage II results and again noted significant transmission

distortion to female probands (p = 0.04, OR = 1.47) but not male probands (p = 0.18), however the over-transmitted allele was the A allele, in contrast to the US samples.

The joint distribution of test statistics for SNP analyses from stages II and III (US case-control and Bulgarian trios, respectively) found individual SNP associations at all four genes ($p_{joint} < 0.05$), including 7 *SLC6A3* loci (Table 9).

We next tested the putative epistatic interactions from the US sample in this cohort. Interaction tests were limited to the 29 SNP pairs where epistasis was detected in the US sample at $p \leq 0.10$ or better using a conditional logit model. Remarkably, 7 of these 29 interactions (24.1%) were significant ($p < 0.05$) in this independent family-based cohort. Consistent with the patterns observed in the US sample, interactions with *SLC6A3* loci were replicated with each of the other three genes (e.g. $p < 0.05$ for the same locus pairs in both samples when analyzing *SLC6A3*DRD3, SLC6A3*SLC18A2*, and *SLC6A3*COMT*). One *DRD3*SLC18A2* interaction was also significant in both samples. Table 9 lists all pairs of loci where at least a trend ($p < 0.10$) was detected in both samples (Table 9).

To interpret the results from our interaction tests we performed simulations of our analysis design. Using permutation and rejection sampling methods, we emulated the complicated multi-stage design employed here. The simulation results suggest it would be unusual to obtain 7 or more "replicated interactions", such as in stage III above. From the simulations we estimate the probability of this event to be roughly one in a thousand (0.0013 ± 0.00071). Similarly, we estimate the results of finding the initial 29 interaction "trends" ($p \leq 0.10$) in stage II to also be rare, despite the much larger number of tests (0.0078 ± 0.0055).

Table 8 Associated SNPs at *SLC6A3, DRD3, SLC18A2,* and *COMT*: joint analyses of US and Bulgarian samples

					US Cases / Controls (478 / 501)				Bulgaria Trios (659)				Joint Analyses	
Gene	SNP	Position	Nuc	HapMap Freq CEU/JPT/YRI	Freq	Z_1	P_1	OR	Freq	Z_2	P_2	OR	Z_{joint}	P_{joint}
SLC6A3	rs12516948	1444369	A	.67/.81/.56*	0.67	-2.5	0.01	0.79	0.65	-1.3	0.21	0.90	-2.6	0.009
SLC6A3	rs6347	1464412	A	.72/.93/.38	0.71	1.1	0.26	1.12	0.75	1.7	0.10	1.17	2.0	0.046
SLC6A3	rs464049	1476905	C	.51/.63/.74	0.52	2.5	0.01	1.25	0.53	2.5	0.01	1.22	3.5	0.0004
SLC6A3	rs456082	1483515	T	.70/.51/.46	0.77	2.2	0.03	1.27	0.77	1.7	0.09	1.17	2.7	0.007
SLC6A3	rs463379	1484164	C	.70/NA/.47	0.77	2.1	0.04	1.26	0.77	1.8	0.07	1.20	2.7	0.006
SLC6A3	rs403636	1491354	G	.79/.64/.78	0.85	-2.0	0.05	1.27	0.85	-1.5	0.15	0.85	-2.4	0.017
SLC6A3	rs3756450	1501148	T	.84/.57/.50	0.87	1.7	0.09	1.27	0.85	2.1	0.04	1.27	2.7	0.007
DRD3	rs7625282	115364217	A	.73/.76/.72	0.76	2.5	0.01	1.26	0.74	0.6	0.52	1.06	2.1	0.033
SLC18A2	rs363393	118995757	A	.83/1.0/1.0	0.81	1.1	0.28	1.10	0.84	1.9	0.06	1.22	2.1	0.033
SLC18A2	rs363338	118999379	T	.69/.24/.32	0.66	2.2	0.03	1.26	0.67	0.7	0.46	1.06	2.0	0.043
SLC18A2	rs363227	119016556	C	.89/.71/.68	0.87	1.4	0.17	1.15	0.87	1.5	0.13	1.21	2.0	0.041
COMT	rs174696	18327730	T	.81/.54/.34	0.79	2.0	0.05	1.24	0.84	1.3	0.19	1.16	2.3	0.029
COMT	rs165815	18334027	T	.88/.65/.41	0.83	1.8	0.07	1.26	0.78	1.4	0.15	1.15	2.3	0.017

*SNPs listed if joint distribution of test statistics from stages II and III resulted in $p_{joint} < 0.05$. Nuc = nucleotide of common allele. Allele frequency (freq) of the common allele is given. Allele frequencies from HapMap data for Caucasians (CEU), Asians (JPT), and Africans (YRI) are given. Direction (sign) of the Z score is provided for the common allele (e.g. Z = -2.5 indicates that the less common allele confers risk). OR = odds ratio for common allele. *Reference data from Applied Biosystems AoD submission for Caucasian, Japanese, and African-American populations (www.ncbi.nlm.nih.gov/SNP).*

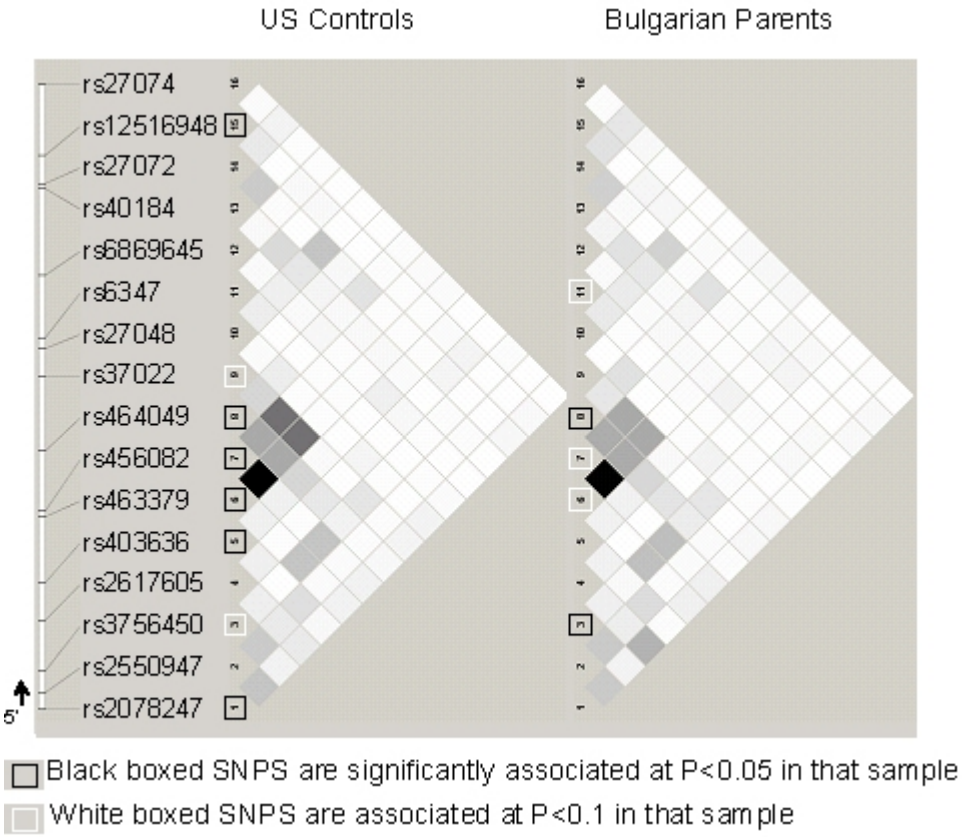

Figure 6 Linkage disequilibrium among SLC6A3 SNPs

Linkage disequilibrium (LD) patterns among all SLC6A3 SNPs genotyped in the US and Bulgarian samples (16 SNPs were common to both samples). LD values between pairs of SNPs (r^2) are indicated, and associated SNPs ($p < 0.10$ and $p < 0.05$) are shown.

Table 9 Noteworthy epistatic interactions *at SLC6A3, DRD3, SLC18A2*, and *COMT*

		Stage II: US Case-Control		Stage III: Bulgarian Trios		Combined Results	
Genes	**Loci**	**Interaction P-value†**	**Perm.^ P-value**	**LL_{Diff}‡**	**P-value**	**χ^2_4††**	**P-value**
SLC6A3*COMT	rs464049*rs174696	0.005	0.001	5.2	0.023	18.1	0.001
	rs464049*rs165815	0.001	0.001	2.5	0.101	17.7	0.001
	rs463379*rs174696	0.091	0.013	6.7	0.009	14.1	0.007
	rs456082*rs174696	0.069	0.009	5.9	0.015	13.7	0.008
SLC6A3*SLC18A2	rs6347*rs363338	0.03	0.023	7.9	0.005	17.6	0.001
SLC6A3*DRD3	rs463379*rs10934256	0.047	0.063	5.3	0.021	13.8	0.012
	rs12516948*rs6280	0.099	0.005	3.8	0.052	10.5	0.033
DRD3*SLC18A2	rs1800828*rs363227	0.026	0.017	4.4	0.036	13.9	0.008
	rs1800828*rs929493	0.051	0.021	3.4	0.065	11.4	0.022

*Epistatic interactions results between stages II and III. The first column lists the pairs of genes at which interactions were detected. The second column lists the corresponding pairs of SNPs. For example, rs464049*rs174696 denotes a SNP at SLC6A3 interacting with a COMT SNP. Only interactions detected from both samples at $p \leq 0.10$ are listed. †P-value for interaction term above main effects in logistic regression (see (Macgregor & Khan, 2006)). ^Perm. = Permutation, p-value from 1,000 iterations permuting case-control status. ‡Difference in -2* log likelihood of full model including an interaction term and a reduced model including only main effects (distributed as a χ^2_1.). ††Test statistic from combining p-values from US and Bulgarian analyses (χ^2_4).*

4.4.6 Stage IV: Functional Analysis

We selected rs3756450 and rs464049 for further analyses of allele specific functional effects as these SLC6A3 SNPs were associated individually with risk for schizophrenia in both samples and featured prominently in the epistatic analyses.

We performed electrophoretic mobility shift assays (EMSA) using nuclear extracts from SHSY-5Y cell line (Figure 8). Both allelic probes at rs3756450 generated DNA-protein gel shift bands. Addition of 50X fold unlabeled oligonucleotides probes for each allele inhibited formation of the gel shift bands, demonstrating specificity for these oligonucleotide sequences. We observed three distinct DNA-protein gel shift bands for the T allelic probe at rs3756450. In contrast, the C allelic probe at rs3756450 annealed to

only two of the three bands, indicating allele specific difference in DNA-protein complex formation. The result was replicated in two additional experiments, including one in which two fold excess of nuclear extract was added for assays with the C allele (see Figure 8). In contrast, no allele specific DNA-protein gel shift bands were observed at rs464049, though bands common to both alleles were noted (data not shown).

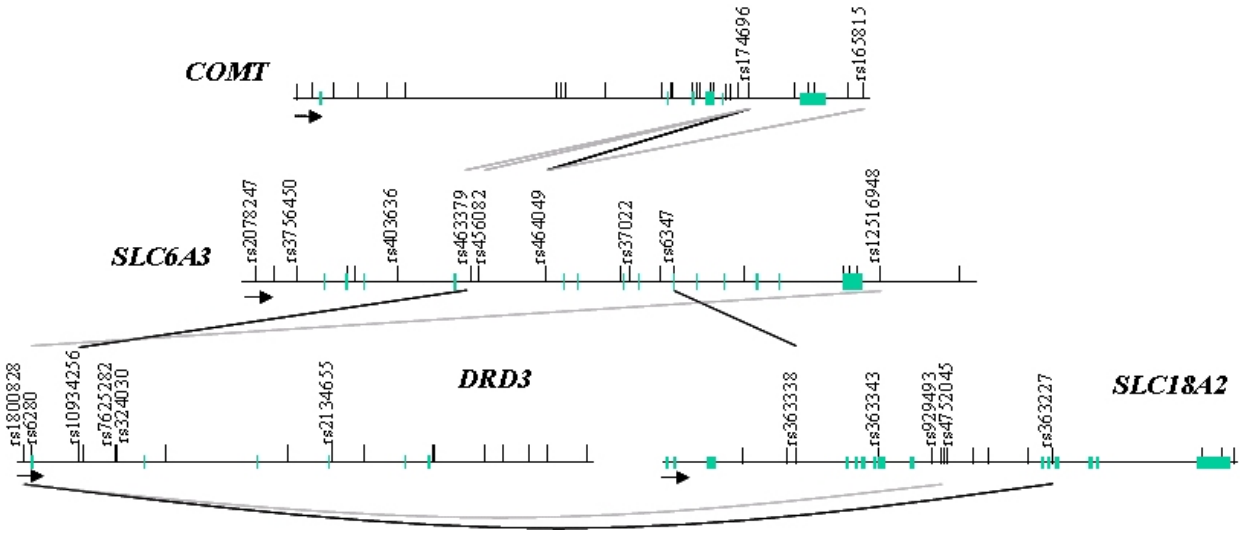

Figure 7 Epistatic interactions at *SLC6A3*, *DRD3*, *SLC18A2*, and *COMT*.

The genomic organization of all four genes retained from stage I analyses is shown. Boxes extending below the horizontal line indicate exons and black tick marks represent all SNPs analyzed in the US and Bulgarian samples. The SNPs retained for epistatic interactions (i.e. SNPs where $p \leq 0.10$ for main effects) are listed. Gray lines indicate epistatic interactions at $p < 0.10$ in both the US and Bulgarian samples, bolded black lines indicate significant interactions in the US as well as the Bulgarian samples at $p < 0.05$.

Since rs3756450 is localized 5' to the putative promoter region of SLC6A3, we also evaluated its effect on transcription. Dual-luciferase assays were conducted using four clones from CEPH individuals whose genotypes were known (two constructs for each allele, see Figure 8). Significant promoter activity was present in all constructs, compared with the promoterless construct. In addition, promoter activity was

significantly different between constructs carrying C and T alleles (Student's t-test, t = 10.32, 5 d.f., $p < 0.0001$; Figure 9).

Figure 8 Electorphoretic mobility shift assays of rs3756450

*Nuclear extracts from SHSY-5Y cells were incubated with labeled probes. The labeled probe for the T allele was loaded in the first three lanes, and the labeled probe for the C allele in the next three lanes. Unlabeled competitor oligonucleotides were included in 50-fold molar excess in lanes 3 and 6. Lanes 1 and 4 indicate the migration of the labeled probe without the nuclear extract . * indicates altered band shift pattern for T-allele of rs3756450 (lane 2) with respect to C-allele (lane 5), despite two fold excess of nuclear extract added to lanes 5 and 6.*

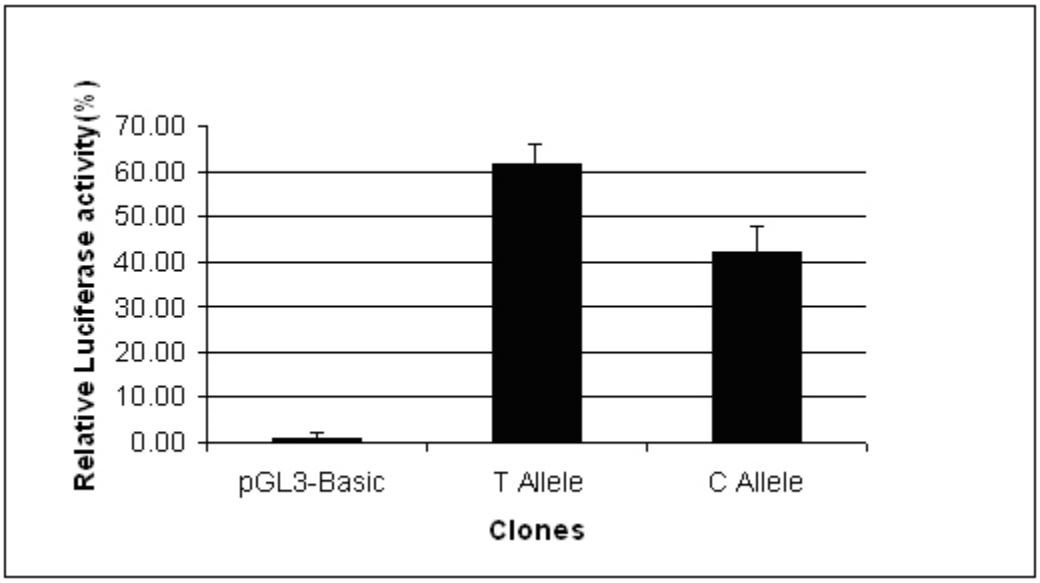

Figure 9. Promoter assays using rs3756450

Promoter activity in a dual-luciferase assay system for constructs containing either the C or T allele at rs3756450, but identical at all other bases.

4.5 DISCUSSION

Our systematic multi-stage approach yielded novel SNP associations and replicated epistasis between four dopaminergic genes, *SLC6A3*, *DRD3*, *COMT* and *SLC18A2*. We also noted plausible allele specific functional effects *in vitro* for one of the associated *SLC6A3* SNPs (rs3756450). Three of these genes have been frequent targets in previous schizophrenia association studies (*DRD3, COMT, SLC6A3*), yet prior studies have not provided definitive evidence for or against associations. Overall, the *SLC6A3* associations were most striking. More than a third of test statistics for stage II analyses involving *SLC6A3* SNPs were significant. The median trends test statistic among 18 SNPs was 2.26, indicating a significant shift towards small p-values compared with expectations. When we sought evidence for epistasis, SNPs at *SLC6A3* also dominated the list. Two *SLC6A3* SNPs (rs3756450 in the 5' upstream and rs464049 at intron 4) were involved in 41.2% of the interactions in the US samples. When we evaluated an independent Bulgarian sample, both these SNPs were again associated. Though the dopamine transporter has long been a target for genetic association studies of schizophrenia (reviewed byBannon, Michelhaugh, Wang, & Sacchetti, 2001), most reports have focused on a variable number tandem repeat (VNTR) polymorphism localized to 15th exon (Gamma et al., 2005), but meta-analysis does not suggest an association (Gamma et al., 2005). A previous analysis of the *SLC6A3* 3' VNTR in a subset of the Bulgarian families also was not significant (Georgieva et al., 2002). Associations with other *SLC6A3* polymorphisms have been reported, including significant associations in the 5' region near the promoter (Keikhaee et al., 2005; Khodayari et al., 2004; Stober et al., 2006).

At *DRD3*, the present associations are consistent with our previous report, which analyzed a smaller set of US cases and a different group of control samples (Talkowski, Mansour et al., 2006). They follow in a long line of studies that have targeted rs6280, a non-synonymous functional polymorphism (Jonsson et al., 2004). More recent studies have shown associations with other variants in both the 5' and 3' regions of the gene (seeTalkowski et al., 2007).

A functional exonic SNP (rs4680, Val/Met) has been the focus of numerous association studies at *COMT*, but the results have not been replicated consistently (M. D. Fallin et al., 2005; J. B. Fan et al., 2005; Glatt et al., 2003a; Munafo, Bowes, Clark, & Flint, 2005; H. J. Williams et al., 2005; reviewed byH. J. Williams, Owen, & O'Donovan, 2007). Associations with haplotypes including rs4680 have been reported recently among Chinese and Ashkenazi Jewish samples (T. Li et al., 2000; Shifman et al., 2002). The latter reported on a haplotype of large effect size comprising three SNPs spanning the gene (rs737865–rs4680–rs165599), and the association was more significant among women. This haplotype was later found to be associated with decreased COMT mRNA levels in the human brain (Bray et al., 2003). Gender specific associations have also been detected with a SNP in this haplotype (rs737865) in late onset Alzheimer's disease with psychosis (Sweet et al., 2005). Our US samples revealed a gender related association between schizophrenia and rs737865 consistent with the Shifman results (OR = 1.34). By contrast, our analyses of the Bulgarian sample found over-transmission of the opposite risk allele (A allele), matching the results of Sweet et al. (42). Unlike the other three candidates, to date only one small association study of Japanese families at *SLC18A2* has been conducted (Kunugi et al., 2001).

The epistatic interactions suggest a susceptibility model in which variations at *SLC6A3* are important determinants of schizophrenia susceptibility, with additional risk due to variants at *SLC18A2, DRD3*, and *COMT*. This model is appealing because all four proteins regulate synaptic dopamine concentrations, and there are plausible functional relationships between these genes. The dopamine transporter (DAT) controls both the intensity and duration of dopamine actions at synapses by modulating reuptake into the pre-synaptic nerve terminal (Sotnikova, Beaulieu, Gainetdinov, & Caron, 2006; Torres, 2006). Because DRD3 may function as an autoreceptor (Sokoloff et al., 1992; Sokoloff, Giros, Martres, Bouthenet, & Schwartz, 1990), it is reasonable to suggest molecular interactions between DRD3 and DAT. Indeed, DRD3, as well as the dopamine D2 (DRD2) receptor subtypes can regulate DAT function (Zahniser & Doolen, 2001; Zapata & Shippenberg, 2002). However, the molecular details of this 'cross talk' are not known. Since VMAT2, the protein encoded by *SLC18A2*, mediates the transport of dopamine into synaptic vesicles, molecular interactions between VMAT2 and DAT following DAT mediated reuptake of dopamine into pre-synaptic terminals are possible and require investigation. Finally, COMT is a key enzyme regulating synaptic dopamine levels through catabolism (Napolitano et al., 1995). Common homeostatic mechanisms may thus regulate COMT and DAT.

EMSA analyses suggest specific bandshift patterns using rs46049 probes in neuroblastoma cell lines. More intriguing allele specific effects were observed with rs3756450, which is localized upstream to the core promoter sequences (Kelada et al., 2005). Our results suggest a putative transcription factor that either has differential affinity for the rs3756450 alleles or binds to rs3756450T, but not rs3756450C.

Furthermore, luciferase promoter assays suggest significant differences in promoter activity for alleles of this SNP. Thus sequences flanking rs3756450 may represent a novel promoter domain for *SLC6A3*.

There are some limitations to our association analyses. Though the SNP selection in stage I was more extensive than past studies, more comprehensive coverage would have been desirable for several genes, particularly *MAOA*, *MAOB,* and *DDC*. The samples available for stage I analyses were also limited. We estimate only 41.3% power to detect an effect size of 1.5, so type II errors were possible and undetected liability loci could be present at genes that were not carried forward in stage II. Similarly, our family based US samples had limited power to replicate other reported associations. Our tests of epistasis were relatively conservative as we considered only locus pairs with evidence of a main effect. Evaluation of much larger samples would be required to conduct an exhaustive analysis of all potential interactions across a larger network of dopaminergic genes.

Our study design was intended to first identify promising susceptibility targets and then test these targets as comprehensively as possible. Spurious associations arising from population substructure are unlikely to account for the *SLC6A3* results, as significant associations were detected in both of the family-based samples. Genomic control analyses also did not detect meaningful population substructure and no corrections were necessary. To limit false positive results, we employed three independent samples, analyzing them independently and jointly. We also simulated our study design and empirically determined the probability of obtaining similar results to the epistatic interactions. These simulations suggest that both our stage II and stage III

interaction findings are unlikely under the null hypothesis (about eight in one-thousand and one in one-thousand for stages II and III, respectively).

In conclusion, our analyses of eighteen dopaminergic genes among over 3,000 participants indicate that variants at *SLC6A3*, *DRD3*, *COMT,* and *SLC18A2* individually and jointly confer risk for schizophrenia. Our findings propose a model for schizophrenia risk in which risk conferred by *SLC6A3* variations could be modified by variants at *DRD3*, *COMT,* and / or *SLC18A2*.

4.6 ACKNOWLEDGEMENTS

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5.0 STUDY #3: CONVERGENT PATTERNS OF ASSOCIATION BETWEEN PHENYLALANINE HYDROXYLASE VARIANTS AND SCHIZOPHRENIA IN FOUR INDEPENDENT SAMPLES

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5.1 ABSTRACT

Recessive mutations in the Phenylalanine hydroxylase (PAH) gene predispose to phenylketonuria (PKU) in conjunction with dietary exposure to phenylalanine (Phe). Previous linkage and association studies have suggested *PAH* variations that could confer risk for schizophrenia, but comprehensive follow-up studies have not been reported. We analyzed 15 common *PAH* "tag" SNPs and 3 rare exonic variations among four independent samples (total n = 5,414). The samples included two US Caucasian cohorts (260 trios, 230 independent cases, 474 controls), a Bulgarian sample (659 trios), and an African-American sample (464 families, 401 controls). Analyses of both US Caucasian samples revealed significant associations with five SNPs (uncorrected $p < 0.05$); most notably the common allele (G) of rs1522305 from case-control analyses ($z = 2.99$, $p = 0.006$). This SNP was independently replicated in the Bulgarian cohort ($z = 2.39$, $p = 0.015$). A non-significant trend was also observed among African-American families ($z = 1.39$, $p = 0.165$), and combined analyses of all four samples were significant (rs1522305: $\chi^2 = 23.28$, 8 d.f., $p = 0.003$); rs10860935 was also nominally significant from the combined results ($p = 0.05$). Case-control analyses in African-Americans were restricted to three exonic variants K274E, L321L, and N426N detected an association with the common allele of L321L ($p = 0.047$, OR = 1.46). Rare alleles were not different between groups at these variants. Our analyses suggest several associations at *PAH*, with consistent evidence for rs1522305. Further analyses, including additional variations and environmental influences such as phenylalanine exposure are warranted.

5.2 INTRODUCTION

Phenylalanine hydroxylase (PAH) catalyses the conversion of phenylalanine (Phe) to tyrosine. This reaction is the rate limiting step in the synthesis of catecholamines and accounts for approximately 75% of the disposal of dietary Phe. The gene encoding PAH is localized to chromosome 12q23.2, contains 13 exons, and the genomic sequence spans approximately 79.3 kilobases. *PAH* is expressed in the liver and kidney.

Mutations in *PAH* can lead to phenylketonuria (PKU) in the presence of a diet that includes Phe. PKU manifests as mental retardation (MR), associated with peculiarities of gait and posture, eczema, epilepsy, light pigmentation, cataracts, brain calcification and a 'mousy' odor (Følling, 1934). These manifestations have been attributed to hyperphenylalaninemia resulting from impaired PAH activity. Early post-natal and long term use of a low Phe diet enables near normal cognitive development (Donlon, Levy, & Scriver, 2004). PKU is inherited as an autosomal recessive disorder, with an average birth incidence of 1 / 10,000 in European populations. Despite the increased frequency of several rare mutations in African-Americans compared to Caucasians, the incidence of PKU in U.S. African Americans is about one-third that in Caucasians (National Institute of Child Health and Human Development). The aggregate mutant allele frequency in these groups is estimated at 0.01. There is considerable allelic heterogeneity, with over 500 catalogued mutations leading to a spectrum of disease ranging from benign hyperphenylalaninemia to classical PKU (www.**pahdb.mcgill.ca**) (Scriver et al., 2003). Genetic heterogeneity is also present, as PKU can occur due to mutations in tetrahydrobiopterin (BH4), an essential PAH co-factor (Thony & Blau, 2006).

Penrose first suggested co-segregation of psychiatric illnesses and PKU, raising the intriguing possibility that *PAH* mutations may contribute to psychopathology other than MR (Penrose, 1935). Studies to explore this hypothesis have been conducted among PKU probands and their relatives, as well as psychiatric patients and their relatives, particularly schizophrenia patients. The severe MR observed among individuals with untreated PKU would preclude a diagnosis of schizophrenia using current criteria, though some case reports with such co-morbidity have been published in the past (Fisch, Hosfield, Chang, Barranger, & Hastings, 1979). More recent case-reports suggesting co-occurrence of PKU among individuals with psychoses have also been published (Shiwach & Sheikha, 1998). A large scale survey among institutionalized psychotic individuals did not detect any individuals with PKU (Cares, 1956). On the other hand, early studies of schizophrenia patients found elevated fasting Phe levels, as well as abnormal responses to Phe tolerance tests (Poisner, 1960), suggesting that some schizophrenia patients could be carriers of mutant *PAH* alleles.

Recent genetic studies have investigated a connection between PAH polymorphisms and increased susceptibility to schizophrenia. Sobell et al. first examined two point mutations (R408W and IVS12nt1) known to be associated with PKU in a case-control study design (190 schizophrenia cases, 336 controls), but did not detect a significant association (Sobell, Heston, & Sommer, 1993). A linkage study of three quantitative traits in a sample of European and African-American schizophrenia affected siblings identified modest evidence for linkage with a marker at 109.5 cM overlapping *PAH* (LOD = 2.12). Linkage with negative symptoms bolstered linkage evidence somewhat for this sample (LOD score = 2.97 at 104 cM), as well as an association

between this marker and schizophrenia (Wilcox, Faraone, Su, Van Eerdewegh, & Tsuang, 2002). A series of studies previously conducted by Dr. Mary Richardson and colleagues have suggested associations between several *PAH* mutations and psychiatric illness among African-Americans but not Caucasians (M.A. Richardson et al., 1999a) (M.A. Richardson et al., 1999b) (Chao & Richardson, 2002). Richardson and colleagues also reported on 9 exonic variants at *PAH* among 123 psychiatrically ill individuals and 34 controls (M. A. Richardson et al., 2003). One exonic variant (K274E) was noted among African-Americans and was over-represented among schizophrenia patients (cases: 4/24; controls: 1/13). The K274E mutation was associated with altered Tyr levels following a Phe loading test. Finally, a recent study detected linkage between short tandem repeat polymorphisms near *PAH* in an island population from Palau when mothers of schizophrenia patients were treated as the affected generation (Devlin et al., 2007). These results are intriguing, because they suggest maternal-fetal interaction in schizophrenia genesis. If true, such a mechanism might account for variability in conventional association and linkage analyses.

Published studies suggest a link between common and / or rare *PAH* polymorphisms and schizophrenia. To investigate this hypothesis, we evaluated 18 *PAH* variations in four independent samples. Our analyses included 15 common polymorphisms and three additional exonic variations reported on previously (M. A. Richardson et al., 2003).

5.3 METHODS

5.3.1 Study design

We tested the hypothesis that common and/or rare *PAH* variations increase risk for schizophrenia (SZ). We analyzed 15 single nucleotide polymorphisms (SNPs) that tagged common variations in Caucasians (Figure 10, details below). These SNPs were evaluated in four independent samples of either European or African-American ancestry. We also selected three variations based on published analyses with psychosis that were monomorphic in Caucasians but polymorphic in African-Americans (K274E, N426N, and L321L, referred to as 'rare variants' herein for clarity) (M.A. Richardson et al., 1999a), (M.A. Richardson et al., 1999b), (Chao & Richardson, 2002). Our primary study included only SNP based analyses, first in each sample individually then combined across samples. Associations with the 'rare variants' were conducted next, followed by exploratory analyses to evaluate covariates such as gender and maternal genotypes.

5.3.2 Samples

US: Unrelated patients were recruited at Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania and surrounding regions (n = 490). Diagnoses were based on the Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994), supplemented by medical records and informant interviews. Consensus DSM-IV diagnoses of schizophrenia or schizoaffective disorder were assigned by board-certified psychiatrists / psychologists following review of all these sources of information. Both parents of 260

patients were ascertained for family based analyses, but diagnostic evaluations were not conducted for the parents (260 trios). Control DNA samples were collected from the cord blood of 474 unscreened Caucasian neonates born at Magee-Women's Hospital, Pittsburgh, PA. Only ancestry and gender was available for these samples.

Bulgaria: schizophrenia patients and their parents were recruited in Bulgaria as described previously (Kirov et al., 2004). Diagnoses among probands were made according to DSM-IV criteria, following assessment by a psychiatrist using the Schedules for Clinical Assessment in Neuropsychiatry (Wing JK, 1990 Jun) , which has been validated for use in the Bulgarian language, and inspection of hospital discharge summaries. All patients and their parents received written information on the project and signed an informed consent form. The Bulgarian sample included 659 trios (total n = 1,977). Probands were diagnosed with schizophrenia (n = 576) or schizophreniaA (n = 83).

African Americans: African-American patients and their parents were ascertained as part of an ongoing collaborative study to investigate risks for schizophrenia in an African-American sample (Aliyu et al., 2006). Families were chosen for genotyping from the overall consortium and analyses were carried out based on phenotype data as of January 19th, 2008. The sample was composed of 464 total families ascertained for both linkage and association studies, including 73 complete trios (proband + 2 parents), 181 "duo + sibs" (proband + 1 parent + unaffected siblings), 122 "case + sibs" (affected proband + unaffected siblings, no parents), 53 affected sibling pairs without parents, 27 affected sibling pairs with 1 parent, 5 affected sibling pairs + both parents, and 3 "duos" (affected proband + 1 parent, no siblings). From these family configurations, most but

not all individuals were informative for family-based association tests. For the three 'rare variants' (see below), 551 African-American cases were contrasted with 401 adult controls. The cases included one patient with schizophrenia or schizophreniaA randomly chosen from each of the 464 families and 87 singleton cases where no parents were available. The controls were screened for absence of psychoses and current substance abuse using the same procedures as the cases (Aliyu et al., 2006). The University of Pittsburgh Institutional Review Board (IRB) approved the study. Approval from appropriate IRBs was also obtained at each collaborating US site. Ethics committee approval was obtained from ethics committees in all regions of Bulgaria where families were recruited. Written informed consent was obtained from all participants, except neonatal controls, in accordance with IRB guidelines.

5.3.3 Polymorphism Selection

We chose tag SNPs to represent all common variations among 60 unrelated Caucasians available in release 20 (phase II, January, 2006) of the International HapMap Project (HapMap, 2003). To accomplish this, we selected all available SNPs within *PAH* and 5 kb of flanking sequence 5' and 3' to the gene. Genotypes were obtained from CEPH samples (US residents collected in 1980 by the Centre d'Etude du Polymorphisme Humain). These participants have ancestry from Northern and Western Europe. Tag SNPs were identified to represent common variation with a minor allele frequency (MAF) greater than 5% in Caucasians using Hclust software (Rinaldo et al., 2005). Hclust computes a similarity matrix from the square of Pearson's correlation (r^2) between allele counts at pairs of loci then uses hierarchical clustering to group correlated SNPs.

We selected a SNP as a tag if the correlation between loci was below a threshold of $r^2 < 0.9$. Thus, 21 SNPs were identified. When SNPs were initially rejected by Applied Biosystems in the assay design (8 SNPs), surrogates were sought. If no surrogates were available, we re-analyzed the dataset to identify another SNP with a lower LD threshold to use as a proxy ($r^2 > 0.8$ between surrogate and failed marker). Using this procedure, only two tag SNPs were not represented at a minimum correlation threshold of $r^2 = 0.8$ in our analyses (rs1281013 and rs1851381). .

Previous research by Richardson et al. suggested associations between several exonic variations and psychosis among African-Americans (M. A. Richardson et al., 2003). Those analyses indicated that the SNPs had minor allele frequencies (MAF) greater than 1% among African-Americans, but had MAF < 0.01 in European Americans. We chose three such variants (K274E, L321L, N426N; referred to in this study as 'rare variants' for clarity) to be genotyped in all of our family samples. An additional set of case-control analyses were conducted for only these SNPs among African-American cases not included in the family based analyses and adult African-American control sample typed exclusively for these polymorphisms.

5.3.4 Genotyping Assays

All 18 variants were included in assays for all four independent samples using the hybridization based SNPlex assay (ABI Biosystems Inc), as described elsewhere (Tobler et al., 2005). The assay utilizes custom designed oligonucleotide pools of up to 48 SNPs, which can be genotyped in a single reaction. The three 'rare variants' were genotyped among the African-American controls using the ABI SNaPshot assay (Applied

Biosystems, Inc). The assay involves a multiplexed PCR reaction followed by single base extension (Mansour et al., 2005). The genomic organization of *PAH* and the selected polymorphisms are shown in Figure 10. All molecular genetic analyses were conducted at the University of Pittsburgh.

5.3.5 Quality control

All genotype assays included duplicated samples and/or CEPH individuals genotyped by HapMap (HapMap, 2003). Negative control samples (water) were also included in each assay plate. A random subset of 34 African-American samples were selected from all individuals found to carry at least one copy of the rare alleles of K274E, L321L, and N426N and individually sequenced to confirm the SNPlex and SNaPshot genotype calls. Tests for Mendelian inconsistencies were conducted in all family-based samples using PEDCHECK (O'Connell & Weeks, 1998) and tests of Hardy-Weinberg Equilibrium (HWE) were carried out for probands, parents, and controls separately in each population using GENEPOP software (version 1.31) (Raymond & Rousset, 1995).

5.3.6 Statistical Analysis

Transmission distortion was analyzed using FBAT software (Laird, Horvath, & Xu, 2000), which can appropriately handle families of mixed configuration such as those in the African-American sample analyzed here. Differences in genotype distributions between cases and controls were evaluated with the Armitage Trends test (SAS software) (Devlin & Roeder, 1999) or Fisher's exact test, as appropriate. Test statistics were

converted to z scores for case-control analyses for ease of comparison regarding risk alleles (i.e. z positive or negative) across samples. We estimated the effective number of independent tests among these SNPs using the statistical package R based on published methods (Conneely & Boehnke, 2007). We estimated the number of effective tests in the Caucasians and African-Americans separately due to the expected differences in LD patterns between these populations. Our analyses suggested 7.9 effective tests in the Caucasians and 12.6 effective tests in the African-Americans. We analyzed each SNP for association in each sample individually. To evaluate evidence against the null hypothesis across the four independent samples, we combined results based on Fisher's combined probability test (Fisher, 1948).

5.3.7 Exploratory analyses

We conducted exploratory analyses to determine if risk conferred by individual polymorphisms was modified by gender. To carry out these analyses, we analyzed allele transmissions to male and female probands separately in family based analyses, and performed logistic regression among male cases / controls and female cases / controls separately.

Previous analyses in an island population detected linkage to the maternal generation of affected schizophrenia cases at 12q23.2 (Devlin et al., 2007). To test the hypothesis of susceptibility due to genetic liability in the maternal generation, we compared allele frequencies for all 15 common SNPs between mothers and fathers in all three samples (Armitage trends test).

5.3.8 Interpretation of statistical significance

We considered an association with schizophrenia significant if (1) an individual SNP test exceeded an alpha threshold of 0.0063 in any Caucasian sample (0.05 / 7.9 tests) or 0.0040 (0.05 / 12.6 tests) in the African-American samples, (2) a nominally significant replication for an individual SNP (and allele) was detected ($p \leq 0.05$ in two or more samples), or (3) combined analyses provided evidence of an association. Exploratory analyses were considered significant only if replication was detected ($p \leq 0.05$).

5.4 RESULTS

5.4.1 Quality Control

All 18 SNPs were genotyped in the Caucasians, but rs124125434 could not be assayed in the African-American samples. The mean genotype call rate was 95% or greater in all four samples for the SNPlex assays and 96.8% in the SNaPshot assays. Using duplicated samples and CEPH individuals to compare with HapMap, we estimated our genotyping accuracy to range between 99.95% - 99.88% in all 4 samples. These data are comparable to HapMap estimates and our previous analyses in these Caucasian samples (Talkowski et al., 2008). We sequenced 34 African-Americans for the three rare variations to confirm their genotype. We found 100% concordance between the sequencing genotypes and SNPlex / SNaPshot genotypes for these individuals.

5.4.2 Linkage Disequilibrium

Linkage disequilibrium (LD) was estimated using Haploview software among unrelated Caucasian controls from the US (n = 474), unrelated parents from Bulgaria (n = 1318), and unrelated African-American parents (n = 367). As expected, pairwise LD (r^2) was similar between Caucasian samples, but differed among African-Americans (Figure 11).

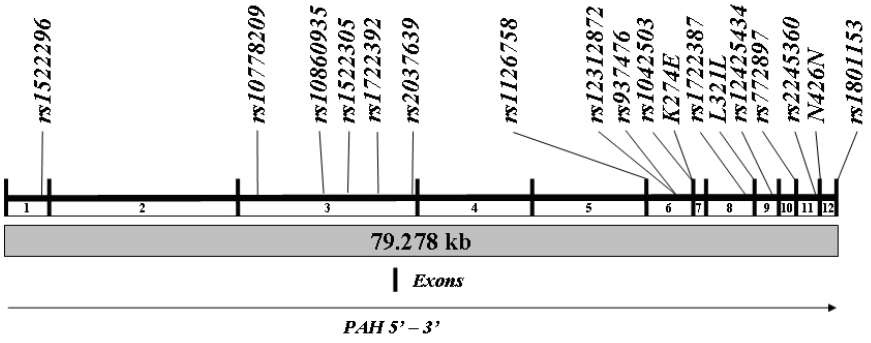

Figure 10 *PAH* genomic organization and variants analyzed

The vertical bars represent exons. The numbers below the line represent the introns. The polymorphisms analyzed are listed above the line.

5.4.3 Primary association analyses

Caucasians: In the US case-control sample (230 cases independent of the trios, 474 controls), two SNPs were associated with schizophrenia (rs1522305, z = 2.74, p = 0.006, OR = 1.64, 95% CI = 1.15 – 2.32; rs12312872, z = 1.98, p = 0.050, OR = 1.34, 95% CI = 1.84 – 0.99; all p-values uncorrected). In the US family sample (260 trios), transmission

distortion was detected with three SNPs, including rs1042503 (z = -2.0, p = 0.05), rs12425434 (z = -2.2, p = 0.03), and rs10860935 (z = 2.3, p = 0.02).

In the Bulgarian families (659 trios), the most significant association in the US case-control analyses (common G allele of rs1522305) was replicated in this independent cohort (z = 2.4, p = 0.015). Three other SNPs were nominally significant (uncorrected p < 0.05; rs2245360, rs937476, rs152296). Transmission distortion that did not reach statistical significance was noted for two SNPs that was consistent with associations in the US sample, namely rs12312872 (Bulgarian p = 0.06, US case-control p = 0.05) and rs10860935 (Bulgarian p = 0.09, US family-based analyses p = 0.02) (see Table 10). The 'rare variants' were monomorphic among all Caucasian samples.

African-Americans: In the African-American family sample, no SNPs were significantly associated with schizophrenia but a trend for over-transmission of the G allele at rs1522305 was noted (z = 1.39, p = 0.167). This is the allele associated in the US and Bulgarian samples and its frequency was similar across samples (US cases 0.898, US cords 0.843, Bulgarian cases 0.875, African-American cases 0.819). All three 'rare variants' (K274E, L321L, N426N) were present at a frequency greater than 1% in the African-Americans. None were significantly over-transmitted to probands, however minor allele frequencies for K274E (0.014) did not enable meaningful analyses of transmission distortion given the size and configuration of the present sample. Case-control comparisons in the African-American samples were therefore conducted for only these three SNPs (551 cases, 402 controls). None of the rare alleles were found to be associated with schizophrenia risk, however a nominally significant association was

detected with the common allele (non-mutant allele) of L321L (p = 0.047, OR = 1.46, 95% CI = 2.14 – 1.0) (Table 11).

5.4.4 Combined analyses

We combined the observed probabilities for each of the four independent samples at each of the 14 SNPs tested across all samples (rs12425434 and each of the three 'rare variants' were not informative for associations in all four samples). As expected from the initial findings in three of the four samples, combined analyses suggested a significant association with the common allele of rs1522305 (χ^2 = 23.28, 8 d.f., p = 0.003). A nominally significant association was also detected with rs10860935 (χ^2 = 15.47, 8 d.f., p = 0.05). Another SNP, rs12312872, was significant among European samples (χ^2 = 12.76, 6 d.f., p = 0.047), but not when African-Americans were included in combined analyses (p = 0.072).

5.4.5 Exploratory analyses

Gender specific associations were detected in the Bulgarian trios with nine SNPs. None were associated in both males and females. Over-transmission to affected male patients was observed for six SNPs, the most significant being rs937476 (p = 0.004, OR = 1.4). Three SNPs were associated among females, most notably rs1522305 (G allele, p = 0.002, OR = 1.84) and rs152296 (G allele, p = 0.007, OR = 1.43) (Supplementary Table 5.1).

Table 10. Association analyses of *PAH* variations

			US Cases / Controls (n = 230 / 474)				US Families (260 Trios)			Bulgarian Families (659 Trios)			African-American Families (n = 464)			Combined Analysis (All samples)	
SNP	Gene Location	Nuc	Case Freq.	Cord Freq	Z_1	P_1	Allele Freq	Z_2	P_2	Allele Freq	Z_3	P_3	Allele Freq	Z_4	P_4	χ_8^2	P_{all}
rs1522296	Intron- 1	G	0.707	0.686	0.77	0.450	0.691	1.61	0.11	0.682	2.23	0.025	0.49	0.01	1	13.39	0.0991
rs10778209	Intron-3	G	0.765	0.751	0.58	0.565	0.740	0.76	0.45	0.716	-0.24	0.813	0.92	-0.58	0.56	4.33	0.8262
rs10860935	Intron-3	T	0.850	0.868	-0.94	0.371	0.856	2.27	0.02	0.837	1.67	0.094	0.66	-0.63	0.53	15.47	0.0506
rs1522305	***Intron-3***	***G***	***0.898***	***0.843***	***2.99***	***0.006***	***0.855***	***0.46***	***0.65***	***0.875***	***2.39***	***0.015***	***0.82***	***1.39***	***0.17***	***23.28***	***0.0030***
rs1722392	Intron-3	C	0.539	0.558	-0.67	0.528	0.557	-0.20	0.84	0.521	-1.38	0.167	0.57	0.01	0.99	5.22	0.7338
rs2037639	Intron-3	G	0.733	0.738	-0.23	0.818	0.739	-1.40	0.16	0.770	0.60	0.550	0.87	-0.27	0.79	5.71	0.6797
rs937476	Intron-6	A	0.586	0.575	0.38	0.722	0.589	0.57	0.57	0.555	-2.07	0.039	0.55	1.39	0.17	11.88	0.1566
rs12425434	Intron-5	C	0.716	0.723	-0.30	0.769	0.708	-2.20	0.03	0.727	-0.22	0.829		N/A			
rs1126758	Exon-6	A	0.557	0.582	-0.90	0.376	0.561	-1.36	0.17	0.579	1.23	0.218	0.8	-0.07	0.95	8.61	0.3763
rs12312872	Intron-6	A	0.858	0.818	1.98	0.050	0.844	0.63	0.53	0.863	1.85	0.064	0.63	-0.77	0.44	14.40	0.0719
N426N	Exon-7	T					monomorphic						0.86	0.67	0.5		
rs1042503	Exon-7	G	0.714	0.727	-0.51	0.614	0.716	-2.00	0.05	0.737	0.44	0.664	0.95	-0.72	0.47	9.46	0.3050
K274E	Exon-7	A					monomorphic						0.98	0.00	1		
rs1722387	Intron-8	G	0.843	0.855	-0.59	0.572	0.845	0.91	0.37	0.841	1.38	0.167	0.85	0.32	0.75	7.29	0.5057
rs772897	Intron-8	G	0.843	0.848	-0.23	0.822	0.842	1.08	0.28	0.834	1.11	0.269	0.82	1.25	0.21	8.70	0.3682
L321L	Exon-9	C					monomorphic						0.94	0.62	0.54		
rs2245360	Exon-11	G	0.642	0.638	0.17	0.870	0.643	0.72	0.47	0.617	-2.18	0.030	0.81	-0.23	0.82	9.24	0.3225
rs1801153	Intron-11	G	0.811	0.787	1.06	0.312	0.821	0.18	0.86	0.739	0.84	0.399	0.39	0.53	0.6	5.50	0.7030

Results from association analyses of 18 PAH variations in four independent samples. SNPs are provided in the direction of PAH transcription 5' to 3'. Nuc = nucleotide for which frequency data are listed. Freq = allele frequency of allele for which nucleotide is provided (common allele). Z = test statistic for common allele (negative = risk conferred by minor allele). Combined analysis using Fisher's method of combining probabilities from independent tests of significance (distributed as χ^2_{2N} statistic).

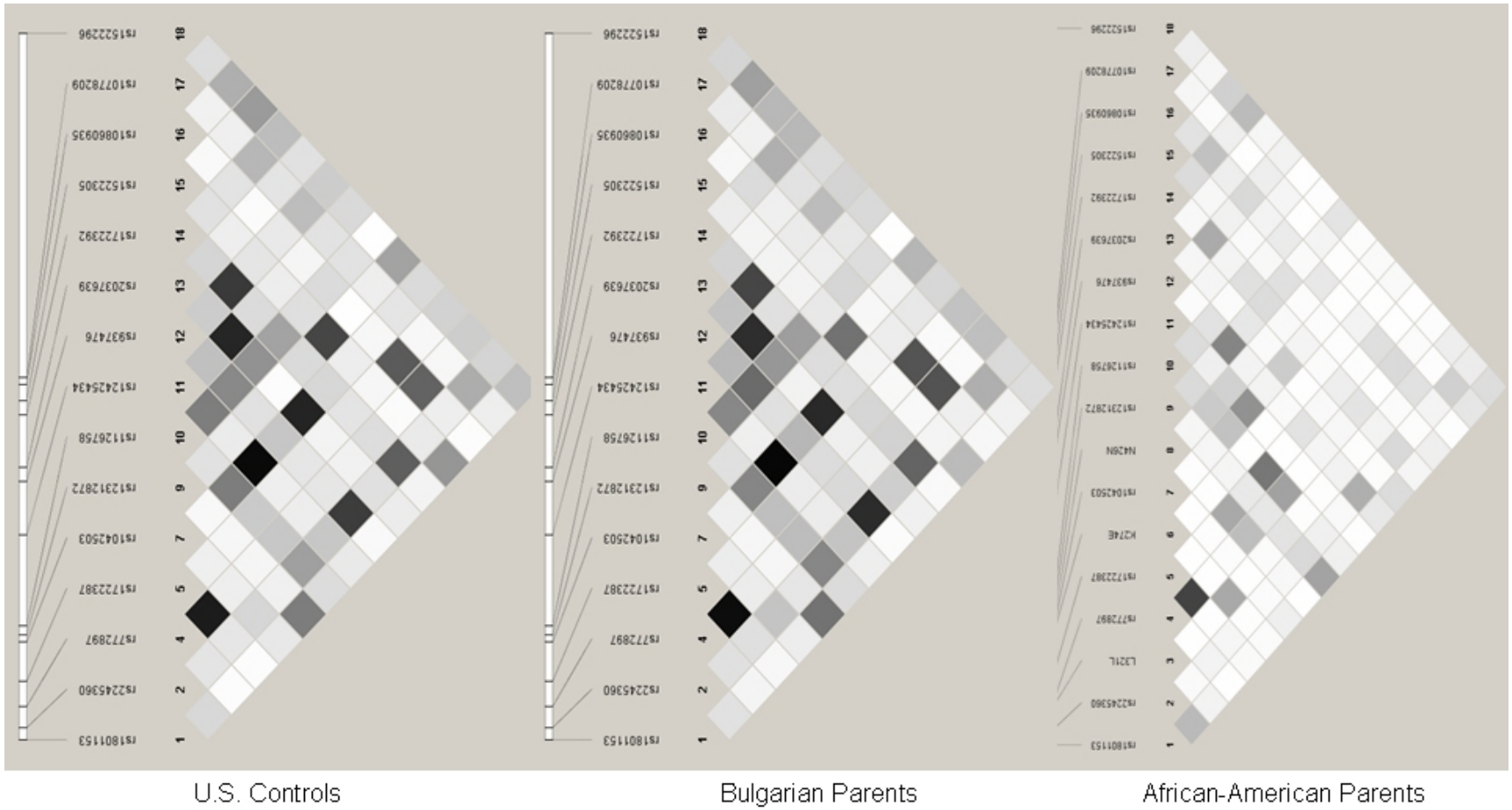

Figure 11 Linkage disequilibrium between *PAH* variants across populations

Linkage disequilibrium (r^2) was estimated between SNPs among (a) unrelated US Caucasian individuals, (b) parents of affected Caucasian probands from Bulgaria, and (c) parents of affected African-American probands.

Replicate analyses in the US and African-American samples detected a significant association with the common allele of rs1522305 when US Caucasian female cases were compared with female controls (US case-control p = 0.05). However, an association was not detected among the US Caucasian trios or the African-American family sample. Consistent replication was also detected between Bulgarian male patients and US male patients ($p \leq 0.05$ in both samples) with rs1042503, rs12425434, and rs2037639. None were replicated among US Caucasian male probands or African-American males. (Supplementary Table 5.1).

We compared the allele frequencies of the 18 common polymorphisms between the mothers and fathers in all three available family samples. No significant differences were found for any of these comparisons (data not shown).

Table 11 Comparison of three PAH variations among African-Americans

		Case Genotype			Control Genotype			Allele Frequencies and association tests			
SNP	Nuc	11	12	22	11	12	22	Case Freq	Control Freq	*Y*	p-value
K274E	1 = A 2 = G	523	16	0	369	17	0	0.985	0.978	1.35	0.246
L321L	1 = C 2 = T	483	52	2	326	57	0	0.948	0.926	3.96	0.047
N426N	1 = C 2 = T	9	138	386	9	83	287	0.146	0.133	0.63	0.428

*Case-control analyses of 3 exonic PAH variations (referred to in text as 'rare variants') among an African-American case-control sample. Nuc = nucleotide. Freq = allele frequency. Y, p-value: results of trends test from distribution of genotypes. *Fisher's exact test p-value: K274E, p = 0.207, L321L, p = 0.017.*

5.4.6 Interpretation of statistical significance

Our analyses found the equivalent of 7.9 effective tests in each individual Caucasian sample and 12.6 effective tests in the African-American samples. There were thus 36.3 effective tests across all four samples for the primary analyses and 132.6 total tests across all primary and exploratory analyses. The associations at rs1522305 fulfilled all three pre-established criteria for significance. The initial analyses in the US case-control sample exceeded the individual experiment correction for multiple testing (uncorrected p = 0.006, corrected p = 0.047) (criterion #1 above). This SNP was significant in two independent samples (US case-control p = 0.006, Bulgarian p = 0.015) (criterion #2), and was associated following combined analyses from all four samples (p = 0.003) (criterion #3). No other SNP associations were robust to correction for multiple testing in individual samples, nor were any other SNPs replicated in more than one sample, although rs10860935 was significant in combined analysis of all samples (p = 0.05) (Table 10).

5.5 DISCUSSION

We tested associations between *PAH* variants and schizophrenia by evaluating tag SNPs to represent all available common *PAH* SNPs among Caucasians, as well as three 'rare variants' previously suggested as risk factors for schizophrenia. We detected several associations of modest effect size in individual samples, with one replicated association in multiple cohorts. The magnitude of the effects detected here were similar

to those reported with other genes in complex disorders (odds ratios 1.10 – 1.50). Simulation studies, as well as analyses of the association between apoE variants and Alzheimer disease suggest that variable patterns of association can be observed in independent samples of varying size, particularly if the primary risk variant is not investigated (Bacanu et al., 2002) (C. E. Yu et al., 2007). Thus, it is often difficult to replicate associations with genetically complex disorders consistently across samples, especially if the magnitude of the association is modest. To reduce the probability of rejecting associations prematurely, we conducted analyses in four individual samples, followed by combined analyses. Using this approach, a consistent association was detected at rs1522305. The association was nominally significant in two of the three Caucasian samples and combining the results across all four samples revealed a significant association. Similarly, exploratory analyses yielded replicable results related to gender between European samples at this locus. Our analytic strategy combined test statistics from multiple independent samples (even those with modest power) in an effort to identify meaningful schizophrenia risk conferred by the same allele that may not reach nominal significance in individual samples.

Prior studies have suggested that *PAH* mutations or exonic polymorphisms may be risk factors for schizophrenia among African-Americans (M. A. Richardson et al., 2003). We evaluated three such variants in all our samples. We detected one nominally significant association with L321L, a synonymous substitution among African-Americans. The associated allele is the common allele, consistent with the results of Richardson et al., however our results failed to support the findings of risk conferred by the rare allele of N426N. These variants appeared to be monomorphic in the Caucasian

samples, although it is possible that rare alleles were present in individuals that failed the SNPlex assays for these SNPs. More comprehensive analyses of other known *PAH* mutations and / or deep sequencing of the region are indicated.

It is not known if allelic variation at the associated SNPs alters PAH activity, so the functional impact of the associations is uncertain. It is possible that the associated SNPs serve as surrogates for unidentified primary risk allele(s). There is modest LD between rs1522305 and two other SNPs, namely rs12312872 and rs1042503 (Figure 11). Analysis of available HapMap data also suggested LD with more remote SNPs, e.g., an intergenic region 100.2 kb 3' to rs1522305 (rs1722400, D' = 0.75, r^2 = 0.52). If the associated SNPs have demonstrable effects on transcription, there are plausible mechanisms for the genetic associations. Hyperphenylalaninemia (HPA) following PAH deficiency can enhance competition between phenylalanine and tyrosine for transport across the blood brain barrier (BBB) (Pardridge & Choi, 1986). Reduced transport of tyrosine across the BBB may decrease catecholamine synthesis (Fernstrom & Fernstrom, 2007). The reduced synthesis may lead to altered dopamine function, a well known mechanism proposed for schizophrenia genesis (Carlsson, 1988) (Snyder, 1973) (Seeman et al., 1976). HPA may also increase Phe catabolism through alternative pathways, such as increased synthesis of phenylethylamine (PEA), a putative psychotogenic compound (Jeste et al., 1981). This hypothesis has been investigated extensively previously, albeit with conflicting results (O'Reilly & Davis, 1994).

Several other lines of investigations may prove helpful in order to further explore the present results. Since current DSM IV criteria preclude a diagnosis of schizophrenia in the presence of MR, it would be of interest to estimate the prevalence of psychoses

among PKU patients who have undergone rigid dietary control. Unfortunately, most published follow-up studies have involved children prior to the modal age at onset for schizophrenia (Weglage et al., 2000) (Corcoran et al., 2005). Interestingly, several investigators have reported that frontal lobe dependent cognitive functions are impaired into young adulthood even among PKU patients who were treated early and aggressively (Welsh, Pennington, Ozonoff, Rouse, & McCabe, 1990) (Diamond, Ciaramitaro, Donner, Djali, & Robinson, 1994) (Corcoran et al., 2005). Similar cognitive impairment has been noted among patients with schizophrenia and their relatives (Gur et al., 2007) (Greenwood et al., 2007). Evaluation of cognitive function among patients with the putative risk alleles may prove insightful in this regard. To follow up Penrose's early analyses, re-examination of psychiatric disorders among obligate carriers of PAH mutations (e.g., parents of individuals with PKU) may also be informative (Penrose, 1935).

The clinical features of PKU are manifested only when individuals with *PAH* mutations consume a diet that includes Phe. The present study did not evaluate such dietary risk factors. Confirmation of a link between schizophrenia and *PAH* mutations or polymorphisms opens the possibility of use of one of a growing number of therapeutic options for treating PKU (including supplementation with biopterin derivatives and large neutral amino acids) to examine their effect on the development of psychiatric disease. A prior linkage study suggested a role for maternal *PAH* variation in pathogenesis (Devlin et al., 2007). We did not find differences in allele frequencies between mothers of Caucasian probands and controls or fathers of the probands at the associated SNPs. This hypothesis needs to be explored further. The mechanism for the gender related

associations noted here is unclear. It is possible that gender serves as a proxy for other variables.

Improvement on the current analyses could be made in future studies by considering a denser set of polymorphisms in African-American samples. The tag SNPs analyzed in the present study represented common variations in Caucasian samples only. Analysis of the Nigerian sample from HapMap suggests that up to 43 SNPs may have been required to comprehensively represent all available SNPs in African-Americans sample (HapMap, 2003). Moreover, the power of our African-American samples was relatively low, owing to both a smaller number of samples and incomplete family configurations. Therefore, further analyses of African-American samples are required. Despite the decreased power in the African-American and US family samples, our combined analyses considered the p-values from each independent sample equally and could be conservative. It is noteworthy that analyses of the joint distribution of test statistics across groups weighted by sample size also suggested a significant deviation from the null hypothesis at rs1522305 (data not shown).

Our analyses of four independent samples of Caucasian and African-American ancestry identified replicable associations between schizophrenia and an intronic *PAH* polymorphism. The functional role for the associated polymorphisms is unknown. It remains possible that risk is conferred primarily by as yet unidentified polymorphism(s). Further analyses of rare exonic variations, population specific tag SNPs for African-Americans, and additional ethnic groups are warranted, preferably in conjunction with environmental risk factors.

5.6 ACKNOWLEDGEMENTS

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6.0 STUDY #4: SHARED GENETIC ETIOLOGY FOR BIPOLAR DISORDER AND SCHIZOPHRENIA? OVERLAPPING RISK LOCI DETECTED FROM ANALYSIS OF 40 DOPAMINERGIC PATHWAY GENES

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6.1 ABSTRACT

Objective: To determine if dopaminergic gene variations are shared etiological risk factors for schizophrenia (SZ), schizoaffective disorder (SZA) and bipolar disorder (BP1). Prior reports suggest dopaminergic dysfunction in SZ/SZA and BP1, as well as a shared genetic etiology between disorders.

Method: We genotyped 431 'tag' SNPs representing all publicly available common SNPs from 40 dopamine genes simultaneously among 526 BP1 cases, 531 SZ/SZA cases, and 477 screened adult controls. Analyses to test for population substructure were conducted and corrections for multiple testing applied.

Results: We found that 60% of all nominally significant SZ/SZA associations and 41% of all BP1 associations were shared ($p < 0.05$). These shared risk loci were from four genes: *DRD3, DDC, MAOB*, and *DRD1IP*. The most pronounced results were at *DRD3;* 9 of the top 20 ranked SZ/SZA SNPs and 7 of the 20 most significant BP1 associations were *DRD3* variants. Gene-based analyses confirmed the *DRD3* results (empirical p-values: SZ/SZA $p = 0.007$, BP1 $p = 0.013$). Diagnosis specific associations were detected with 6 other DA genes in SZ/SZA and 8 other genes in BP1. Several individual SNP tests remained significant after gene-wide correction. No test statistics were robust

to experiment-wide Bonferroni correction at the level of individual SNPs or epistatic interactions.

Conclusion: Our results suggest shared dopamine risk factors for BP1 and SZ/SZA, as well as disorder related associations. Adequately powered replicate analyses are required to further evaluate these results, as well as possible epistatic interactions.

6.2 INTRODUCTION

Since their conception, there has been vigorous debate about the etiological relationship between bipolar I disorder (BP1) and schizophrenia (SZ) (see (Crow, 2008a) for review). The arguments for and against a continuum have focused on psychopathology and familial aggregation, but the issue is unresolved (Brockington & Leff, 1979; Crow, 1990, 2008a; Gershon et al., 1988; Kendell & Brockington, 1980; Kendler et al., 1993; Tsuang, Winokur, & Crowe, 1980; Valles et al., 2000). Recently, genetic association studies have been brought to bear on this question (Craddock et al., 2005, 2006; Craddock & Owen, 2007; Owen et al., 2007). The proposition that BP1 and SZ lie on a phenotypic and etiological continuum, (with schizoaffective disorder, SZA at an intermediate position) would be strengthened if variations or identical risk alleles in the same genes conferred risk to all three disorders. Such overlap has been suggested for several genes, including dysbindin (*DTNBP1*), brain derived neurotrophic factor (*BDNF*), catechole-o-methyltransferase (*COMT*), disrupted in schizophrenia 1 (*DISC1*), the dopamine transporter (SLC6A3), and neuregulin-1 (*NRG1*), to name a few (Goghari &

Sponheim, 2008; Owen et al., 2007; Perlis et al., 2008) (Serretti & Mandelli, 2008). The evidence in support of the shared etiological hypothesis comes from different studies that evaluated overlapping sets of polymorphisms in either BP1 or SZ samples compared to different control groups. The hypothesis could be more comprehensively tested if the same genetic variants were evaluated systematically and simultaneously among BP1, SZ, and SZA cases compared against the same set of control individuals.

Dysfunction in brain dopaminergic (DA) neurotransmission may have a pathogenic role for SZ as well as BP1 (Carlsson, 1988) (Seeman, 1995) (Crow, 1980b) (Crow, 1987) (Goldberg et al., 1999) (Berk et al., 2007). The 'dopamine hypothesis' of SZ / SZA suggests that 'positive symptoms' such as delusions and hallucinations result from DA dysfunction in the mesolimbic and striatal regions, while negative symptoms are a consequence of DA deficits in the prefrontal regions of the brain (Lang et al., 2007). A DA model of BP1 pathogenesis has also been proposed (Berk et al., 2007). The model predicts a cyclical dysregulation of DA transmission, with DA increases during manic phases followed by secondary down regulation and consequent decreased DA neurotransmission during depressive episodes (Berk et al., 2007). These models could implicate a common pathogenic pathway for both disorders, and thus a possible common genetic etiology.

Numerous DA polymorphisms have been investigated in SZ/SZA, and to a lesser extent in BP1, with inconsistent results. Our recent review of DA gene association studies in SZ suggested relatively low power and sparse coverage of common variants in most publications (Talkowski et al., 2007). Since different DA gene variants have

typically been evaluated in each study, it has often been difficult to conduct meaningful meta-analyses (Allen et al., 2008).

We recently proposed a model of SZ/SZA susceptibility centered on four interacting DA genes, namely *SLC6A3* (DAT), *DRD3*, *SLC18A2* (VMAT2), and *COMT* (Talkowski et al., 2008). The findings were derived from an initial screen of eighteen DA genes and the results were replicable in two large Caucasian samples. To address the BP1-SZ continuum hypothesis, it would be necessary to evaluate the same genes for BP1. Our initial screen of DA genes did not adequately represent common polymorphisms that are currently available in public databases (e.g., HapMap), nor did it investigate the entire list of DA genes. Hence we have extended our analysis from the initial set of 18 DA genes to 22 other DA interacting proteins. To comprehensively consider common variants, we selected representative tag SNPs from all publicly available SNPs within these 40 genes. We simultaneously compared BP1 and SZ/SZA samples to an adult control sample that was screened for absence of these disorders. We sought plausible individual and overlapping risk loci for each disorder, with appropriate corrections for multiple comparisons applied.

6.3 METHODS

6.3.1 Samples

BP1 cases: We obtained genomic DNA from 526 patients with BP1 recruited through the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD), a

longitudinal study aimed at improving the treatment for BP1, (Sachs et al., 2003) (Sklar et al., 2008). STEP-BD used a network of eighteen U.S. treatment centers for standardized evaluation and treatment of patients including interview schedules based on the Structured Clinical Interview for DSM-IV (SCID), as well as the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) (Spitzer, Williams, & Gibbon, 1996).

SZ/SZA cases: Unrelated patients were recruited at Western Psychiatric Institute and Clinic, Pittsburgh, PA and surrounding regions (n = 527). Diagnoses were based on the Diagnostic Interview for Genetic Studies (DIGS)(Nurnberger et al., 1994), supplemented by medical records and informant interviews. All participants met consensus DSM-IV diagnosis of SZ or SZA by board-certified psychiatrists / psychologists. Of these, 213 (40.4%) met diagnostic criteria for SZA. Most of these SZ/SZA patients (n = 460) were genotyped in our previous analyses of DA SNPs (Talkowski et al., 2008).

Screened adult controls: An adult control sample, screened for absence of BP1, SZ or SZA was selected (n = 477). As the STEP-BD BP1 sample was recruited across the USA, we utilized control individuals from the Pittsburgh region, as well as a national sample obtained from the publicly available samples deposited with the National Institute of Mental Health Genetics Research Initiative repository (NIMH-GRI).

Pittsburgh controls (n = 168) Members of the community were recruited through random digit dialing by the Pittsburgh University Center for Social and Urban Research (UCSUR), or through web-based advertisements. All participants were self-identified as

Caucasian and screened using DSM IV criteria through the DIGS and other screening tools for absence of BP1, SZ, SZA, substance abuse disorder within the past month, serious medical or neurological illnesses, and mental retardation as defined in the DSM IV. Individuals who reported a first-degree relative with psychoses or BP1 were also excluded. All participants provided written informed consent, according to the guidelines of the University of Pittsburgh Institutional Review Board (IRB).

NIMH-GRI controls (n = 309) Adult controls were obtained from the ongoing genetic analysis and information network (GAIN) initiative project "Linking Genome Wide Association Study of Schizophrenia" (Suarez et al., 2006) (Manolio et al., 2007). Control individuals were screened for ancestry and asked a series of questions regarding medical history, including any previous treatment and/or diagnosis of schizophrenia and/or schizoaffective disorder, any previous treatment and/or diagnosis of bipolar disorder and/or manic depression, and any previous treatment and/or presence of auditory hallucinations and/or delusions (Sanders et al., 2008). Complete details are available on the study website: (www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000021.v2.p1).

6.3.2 Gene selection

We previously identified 15 genes important in DA neurotransmission that had also been evaluated in SZ association studies, as well as 3 genes known to interact with the DA pathway (Talkowski et al., 2007) (Talkowski et al., 2008). In addition to these genes, 22 additional genes that interact with the DA pathway were selected (see Supplementary Table 6.1 for gene descriptions).

6.3.3 SNP selection

We considered all available common HapMap phase II SNPs (rel 22) (HapMap, 2003) from each of the 40 genes, including 5 kilobases (kb) upstream and downstream of the coding sequence (minor allele frequency > 0.05). Additional SNPs were obtained from the SeattleSNPs project where possible (www.pga.gs.washington.edu). We analyzed linkage disequilibrium (LD) among CEPH individuals and selected tag SNPs at a minimum correlation threshold of $r^2 = 0.9$ using a combination of Haploview software (Barrett, Fry, Maller, & Daly, 2005), Hclust software (Rinaldo et al., 2005), and the genome variation server (www.gvs.gs.washington.edu/GVS). For SNPs did not pass the initial Illumina screen for assay design, we identified suitable surrogates, while maintaining the 0.9 correlation threshold. If no such surrogates were available, the correlation threshold was lowered to 0.8. If possible, we chose multiple surrogates for SNPs previously associated with SZ/SZA or BP1, allowing for a level of redundancy at key genes.

6.3.4 Genotyping and quality control

All polymorphisms were genotyped using the hybridization based Illumina GoldenGate Assay (www.Illumina.com) (Steemers & Gunderson, 2007). We included blind duplicates, negative controls (distilled water) and positive controls (17 CEPH individuals genotyped by HapMap) in all assays. Additional confirmation about genotype calls was obtained from 460 SZ/SZA cases previously genotyped for 64 SNPs (Talkowski et al., 2008). Deviations from Hardy-Weinberg Equilibrium (HWE) were evaluated for each

SNP using a global significance threshold of $p > 0.005$, well above what would be expected by chance from analysis of all SNPs.

6.3.5 Statistical analyses

We tested for individual SNP associations, then obtained a summary statistic for all SNPs within a gene, and finally conducted exploratory analyses for epistatic interactions and potentially important covariates such as diagnosis, gender, and age at onset of illness.

We estimated the effective number of independent tests among SNPs within each gene using the statistical package R, based on published methods (Conneely & Boehnke, 2007). Differences in genotype distributions for individual SNPs among cases and controls were evaluated using the Armitage trends test. Gene based tests were used to evaluate all SNPs within each gene simultaneously. The optimal procedure for evaluating multiple single locus tests of association within a gene can vary depending on the number of SNPs genotyped and the correlation structure within the gene (Roeder et al., 2005). Our genes differed widely by the number of SNPs genotyped (n = 1 - 47) and the correlation structure between SNPs was high. For these analyses we conducted the Hotelling's T^2 multilocus association test (R. Fan & Knapp, 2003). Both the asymptotic p-value and empirical permutation p-values are reported. Analyses were carried out using PLINK, version 0.99r (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007). Analysis of epistasis was conducted for all possible gene-gene SNP pairs in each disorder using logistic regression, as previously described (Talkowski et al., 2008). Exploratory analyses were conducted for each SNP to test for meaningful phenotypic covariates of diagnosis (SZ or SZA), gender, and age at onset of illness.

Corrections for multiple tests: Our analyses included some genes with a strong *a priori* hypothesis (e.g. *DRD3*, *DRD2*, *COMT*, and *SLC6A3*, among others) and other exploratory genes such as the dopamine interacting proteins, many of which have never been tested in association studies. We therefore performed corrections for multiple comparisons in our SNP based analyses at two levels: 1) at the gene level, applying correction for the effective number of independent tests within each gene and 2) at an experiment wide level, correcting for all effective independent tests conducted in both disorders (164.5 independent tests per disorder, 329 total tests; uncorrected threshold $p < 0.0003$ per disorder, $p < 1.5 \times 10^{-4}$ for all tests). For gene-based analyses, empirical p-values for each gene were determined by permutation, and Bonferroni correction for 40 independent gene-based tests was applied. Interaction results were also corrected for all effective independent interactions tested (26,024 tests per disorder, 52,048 total; uncorrected threshold $p < 9.6 \times 10^{-7}$). All exploratory analyses were corrected for the total number of effective tests conducted (164.5 tests x 3 variables x 2 disorders = 987 tests).

Genomic control: We tested for population substructure by comparing SZ/SZA cases to controls as well as BP1 cases to controls using a variation of the genomic control (GC) method (Devlin & Roeder, 1999) (Bacanu et al., 2000) (Devlin, Bacanu, & Roeder, 2004). Briefly, these samples were previously genotyped for the current report and another study investigating variations from the circadian pathway (Mansour et al., *in preparation*). In sum 768 SNPs were genotyped from 64 different genes, i.e. 64

generally independent genomic regions. Next, we performed 10,000 iterations of randomly choosing a single SNP from each of the 64 genes to compare between cases and controls, obtaining a distribution of median chi-square tests. The mean value of this distribution was calculated and was divided by the expected median of a chi-square distribution with 1 degree of freedom (0.456). Since control samples were obtained from two different geographic regions, we conducted identical analyses between the two control groups to assess within-group substructure.

Power analysis

We evaluated the power of our sample to detect an odds ratio of 1.5 under a dominant model, or the maximum expected effect size based on previous studies of DA gene variations using similar sample sizes (Talkowski et al., 2007). We tested the assumptions that the risk allele was actually analyzed in our sample or that a surrogate was genotyped at r^2 of 0.9 with the risk loci (similar to our primary analyses). We assessed power using risk allele frequencies of 0.15, 0.4, and 0.9 in the population, and varied the type I error threshold between 0.05 and 0.00015 (i.e., nominal significance and our corrected significance for all tests).

6.4 RESULTS

6.4.1 Quality control

Of 431 SNPs genotyped, 422 were retained for association analysis (9 SNPs failed QC criteria or were rare, i.e. minor allele frequency less than 0.01). The mean genotype call rate was 99.96% across samples. The discrepancy rate in all positive controls (duplicated samples, overlapping samples from previous studies, and CEPH individuals compared with HapMap) was less than 0.01%, a rate comparable to HapMap (HapMap, 2003).

6.4.2 Association tests

Thirty-seven of the 422 SNPs tested reached nominal significance for BP1 (8.8%, uncorrected $p < 0.05$). Tweny-five SNPs were associated with SZ/SZA (6.2%). Of these 25 nominally significant SZ/SZA associations, 15 SNPs (60%) were also associated with BP1. These shared associations occurred at *DRD3* (8 SNPs), *DDC* (5 SNPs), *DRD1IP* (1 SNP), and *MAOB* (1 SNP). These shared loci accounted for 44.7% of all nominally significant associations observed with BP1.

The top ranked association signal was the same *DRD3* SNP / allele in both disorders, rs9868039 (uncorrected $p = 0.0017$ and 0.0032 in BP1 and SZ/SZA, respectively). Nominally significant associations with BP1 were detected following gene-wide correction for the following genes (number of nominally significant associations in parentheses): *DDC* (11 SNPs), *DRD3* (8 SNPs), *DRD1IP* (2 SNPs), *SLC18A1* (2 SNPs), *DRD4* (1 SNP), and *PPP1R9B* (1 SNP). Associations with SZ/SZA

remained significant following gene-wide correction at *DRD3* (10 SNPs), *DRD1IP* (1 SNP), and *SP4* (1 SNP). Nominally significant associations with SZ/SZA were also detected at *DDC* (6 SNPs) and *DRD2* (2 SNPs). These SNPs were not significant after gene-wide correction (40 and 22 SNPs were tested in *DDC* and *DRD2*, respectively), however both *DRD2* associations were direct replications of risk alleles detected by Sanders et al. (rs17529477: $p = 0.039$, rs7131056: $p = 0.048$; Sanders et al. $p = 0.018$ and 0.012, respectively) (Sanders et al., 2008). No SNPs exceeded our experiment-wide significance threshold of $p < 1.5 \times 10^{-4}$. Gene based tests supported the SNP associations, *DRD3* was significantly associated with both SZ/SZA ($p = 0.007$) and BP1 ($p = 0.013$) following permutation when all SNPs were considered. No other gene based tests were significant, nor did the *DRD3* results survive Bonferroni correction.

Our previous analyses suggested associations between SZ/SZA and *DRD3, SLC6A3* (DAT), *SLC18A2* (VMAT2), and *COMT*. In the present analysis, individual SNPs at *DRD3, SLC6A3* and *SLC18A2* were nominally significant with SZ/SZA, but not *COMT*. Only the *DRD3* associations remained significant after correction.

6.4.3 Exploratory analyses

We tested all possible gene-gene SNP pairs in each disorder separately (i.e., only pairs across genes were tested, but not pairs of SNPs within genes). In sum, 175,818 interaction tests were conducted for each disorder (351,636 total tests), the equivalent of 26,024.3 effective tests for each disorder (52,048 total). No pair-wise interactions were significant at this threshold ($p < 9.61 \times 10^{-7}$). The top ranked shared interactions ($p < 0.001$ in each disorder) and disorder specific results ($p < 0.001$) are found in

Supplementary Tables 6.4. and 6.5. Despite a number of nominally significant results, particularly between *DRD3* variants, gender, and psychotic symptoms, none of our analyses of phenotypic variables exceeded chance expectations (Supplementary Table 6.6).

Table 12 Nominally significant gene-based associations

				SNP Associations				Hotelling's T^2	
Significant Association	Gene	Tag SNPs	Effective tests	# SNPs p<0.05 (SZ)	Best SZ p-value	# SNPs p<0.05 (BP1)	Best BP1 p-value	Empiricalp-value (SZ)	Empiricalp-value (BP1)
SZ / SZA and BP1	*DRD3*	20	6.7	10	0.003*	8	0.002*	0.007	0.013
	DDC	40	11.9	6	0.029	11	0.002*	0.391	0.708
	MAOB	8	3.3	2	0.019	2	0.042	0.268	0.296
	DRD1IP	4	1.9	1	0.012*	2	0.008*	0.090	0.063
SZ / SZA Only	*DRD2*	22	8.3	3	0.037	0	0.261	0.433	0.813
	SLC6A3	47	16.9	1	0.045	0	0.118	0.479	0.818
	NEF3	8	3.5	1	0.044	0	0.075	0.342	0.286
	Sp4	3	1.1	1	0.016*	0	0.687	0.105	0.963
	SLC18A2	18	7.7	2	0.041	0	0.095	0.420	0.393
	GRB2	8	3.4	1	0.037	0	0.208	0.373	0.715
BP1 Only	*FREQ*	21	8.1	0	0.056	5	0.011	0.776	0.376
	SLC18A1	22	8.7	0	0.233	2	0.005*	0.761	0.113
	SNAP25	34	13.3	0	0.064	2	0.012	0.334	0.375
	COMT	31	10.6	0	0.217	1	0.050	0.804	0.518
	SNCA	12	4.9	0	0.128	1	0.038	0.753	0.614
	DBH	29	11.2	0	0.124	1	0.045	0.617	0.878
	PPP1R9B	5	1.9	0	0.068	1	0.019*	0.396	0.095
	EPB41	15	4.9	0	0.138	1	0.022	0.818	0.109

*Chr = chromosome. Effective tests = effective number of independent tests. *Significant after Bonferroni correction for 40 gene-based tests. *Significant after gene-wide correction. No SNPs were significant at a level of experiment-wide correction.*

6.4.4 Testing for possible confounds

A series of tests were conducted to see if the overlapping associations described above arose from biased control selection or comparison groups. Based on our genomic control analysis, no meaningful inflation in the test statistics was observed between SZ/SZA cases and controls ($\lambda = 1.08$), nor were differences found between BP1 cases and controls ($\lambda = 0.85$). We also compared our SZ cases to our SZA cases to determine if meaningful differences existed, which they did not ($\lambda = 0.938$). Since our controls were obtained from two different sources, we performed similar genomic control analyses (10,000 iterations comparing SNPs from 64 different genomic regions, see Methods) between controls from Pittsburgh and the NHGRI controls. Nearly identical results to the case / control comparisons were found between the two sets of controls ($\lambda = 1.08$).

Nominally significant associations with both diagnostic groups were detected at 15 SNPs ($p < 0.05$, see Table 13). Two non-significant trends were also observed. To determine if these results were an artifact of the control group, we compared these 17 SNPs between the two control groups. No significant differences were detected ($p > 0.1$ in all analyses; data not shown). To further interrogate our control sample, we obtained allele frequencies for these SNPs from other sources, where available, including: 1) our previously reported association study with SZ/SA using population based neonatal control samples (Talkowski et al., 2008), 2) HapMap (ref), and 3) available Caucasian populations in dbSNP (build 129). Allele frequencies are available in Supplementary Table 6.7. In general, we found that our case frequencies comported well with the independent control frequencies. Qualitative differences were obvious between our control sample and the independent control samples for some, but not all SNPs.

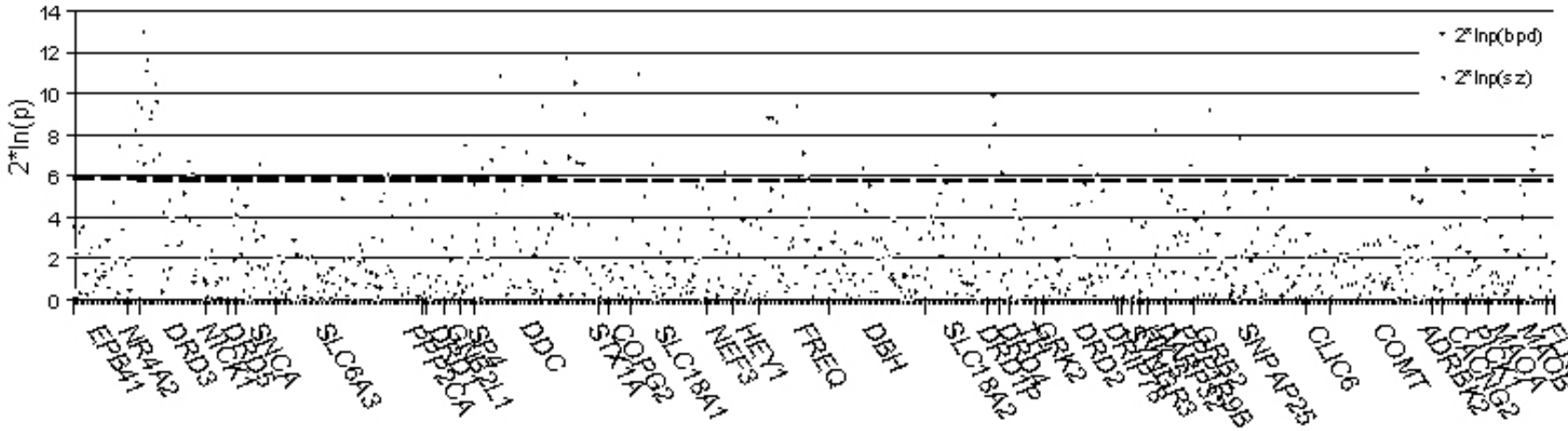


Figure 12 Individual SNP association results across 40 dopaminergic genes

*Trends test p-values for individual SNPs across the 40 genes. The y-axis is -2 x the natural log of the p-value (-2*LN(p)), and the x-axis contains all genes. SNP results are provided in genomic order. **Figure to be updated in color or with different symbols, depending on journal specificity. Dotted line p = 0.05.*

Table 13 Nominally significant associations between individual SNPs and both diagnostic groups

Genomic Data			SNP Association Results					Allele Frequencies		
Gene	SNP	Position	SZ/SZAp-value	SZ OR	BP1 p-value	BP1 OR	N	SZ Case	BD Case	Control
DDC	rs4947535	50499175	0.036	0.82	0.033	0.82	A	0.296	0.295	0.340
DDC	rs745043	50511449	0.029	0.78	0.004	0.73	A	0.206	0.195	0.248
DDC	rs4470989	50530192	0.029	0.81	0.028	0.81	A	0.297	0.297	0.343
DDC	rs3807558	50538516	0.043	0.80	0.008	0.75	A	0.207	0.198	0.246
DDC	rs3779078	50578412	0.042	0.80	0.004	0.74	A	0.205	0.193	0.244
DRD1IP	rs11101694	134996704	0.012	0.73	0.008	0.72	G	0.129	0.127	0.169
DRD3	rs2046496	115317621	0.031	1.21	0.017	1.24	G	0.494	0.502	0.448
DRD3	rs12636133	115322414	0.023	0.81	0.009	0.79	C	0.424	0.416	0.475
DRD3	rs10934254	115324324	0.035	0.83	0.009	0.79	G	0.428	0.417	0.475
DRD3	rs9868039	115329232	0.003	1.30	0.002	1.34	A	0.451	0.458	0.387
DRD3	rs9817063	115329798	0.011	0.79	0.003	0.77	G	0.456	0.447	0.514
DRD3	rs3732790	115329973	0.033	0.82	0.012	0.79	A	0.383	0.374	0.431
DRD3	rs2134655	115340891	0.007	1.31	0.005	1.33	A	0.277	0.280	0.226
DRD3	rs963468	115345577	0.030	0.82	0.008	0.78	A	0.381	0.370	0.429
DRD3	rs7625282	115364217	0.057	0.83	0.073	0.84	G	0.248	0.250	0.284
MAOB	rs2283729	43562986	0.026	1.26	0.042	1.34	A	0.274	0.286	0.231
MAOB	rs6651806	43573908	0.058	1.23	0.052	1.36	C	0.284	0.304	0.243

All SNP based associations that were nominally significant in both SZ/SZA and BP1 are provided. P-values are uncorrected results from Armitage trends test. OR = odds ratio of rare allele. N = nucleotide of rare allele assayed in this study. Allele frequencies for SZ/SZA cases, BP1 cases, and controls provided.

6.4.5 Power analysis

Under a dominant model, the sample had 80.9% power to detect a nominally significant association (OR 1.5) for a risk allele with 15% MAF in the population, using a type I error threshold of 5%. For a risk allele with MAF 40%, power declined to 70.3% under the same assumptions. The sample had approximately 12% power to detect a significance level of $p < 0.00012$, the threshold required to exceed correction for all SNP based analyses.

6.5 DISCUSSION

We systematically evaluated associations between representative, common dopaminergic (DA) gene variants and bipolar disorder (BP1), schizophrenia (SZ) and schizoaffective disorder (SZA). The catalogue of DA genes is dynamic and likely to grow. We included a core group of genes that unambiguously impact DA function, as well as additional DA interacting proteins. Thus, our analyses provided more intensive interrogation of common DA pathway genes and SNPs than previous efforts. We adopted a relatively conservative gene based approach in the present controversy over the type of correction needed for multiple comparisons (WTCCC, 2007).

Our analyses detected multiple associations between dopamine D3 receptor gene (*DRD3*) variants and BP1 as well as SZ/SZA. Both individual SNP and gene based tests were associated with both disorders. Although no single SNP association exceeded

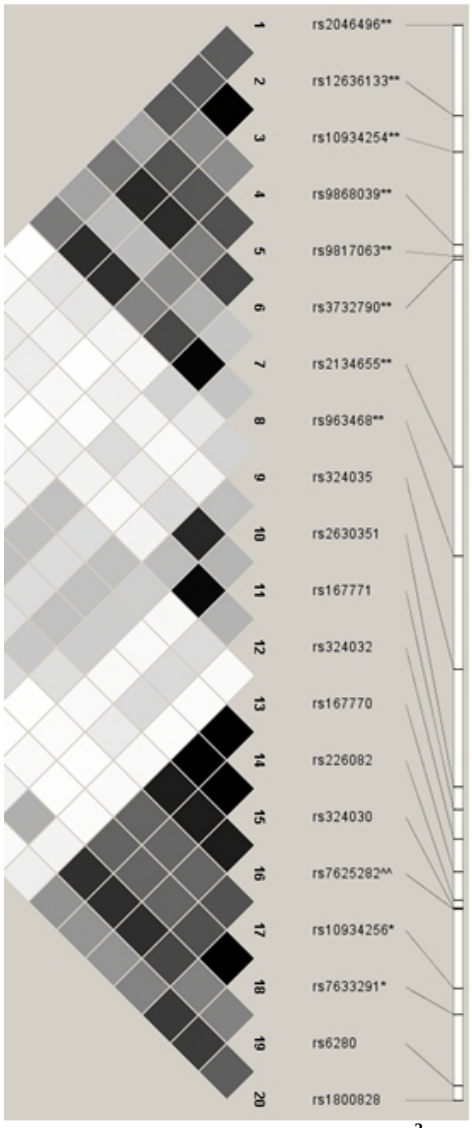
correction for multiple comparisons, 7 of the 20 most significant BP1 associations and 9 of the top 20 SZ/SZA associations were *DRD3* variants. Among the 20 *DRD3* SNPs evaluated, 8 SNPs were associated with BP1 and 10 SNPs were associated with SZ/SZA.

The same allele at rs9868039 of *DRD3* was the most significant SNP in both diagnostic groups across all 422 SNP tests. This SNP was part of a cluster of 8 nominally significant SNPs in both SZ/SZA and BP1 spanning intron 4 to the 3' region of the gene. Linkage disequilibrium between all 8 SNPs was modest (minimum $r^2 > 0.2$, D' > 0.65 between all SNP pairs). Like several previous *DRD3* associations, rs9868039 has unidentified biological function but is localized in the 3' region of the gene approximately 1 kb from the transcription stop site in exon 7 (previously named exon 6)(www.ncbi.nlm.nih.gov). Two SNPs significantly associated with both SZ/SZA and BP1 from this cluster in the current analyses are consistent with our previous findings, however rs9868039 is not among them (rs10934254, rs2134655) (Talkowski, Mansour et al., 2006) (Talkowski et al., 2008). Haplotypes spanning this region were also significantly associated with SZ in an independent study (Dominguez et al., 2007). No SNPs spanning this region were associated with BP1 in two large GWAS studies; however none of our associated SNPs were included in those studies (WTCCC, 2007) (Sklar et al., 2008). Two SNPs within intron 2 (and one additional trend) independent of this 8 SNP cluster were nominally significant in SZ/SZA (rs10934256, rs7633291, rs7625282), but not BP1, consistent with our previous findings (Figure 13) (Talkowski, Mansour et al., 2006) (Talkowski et al., 2008). The second ranked gene in our analyses was dopamine decarboxylase (*DDC*), in which 6 SNPs were associated with SZ/SZA and

11 SNPs were associated with BP1. These results merit replication, as previous studies have not investigated *DDC* extensively (see (Talkowski et al., 2007) for review).

In addition to the shared risk factors, there were diagnostic specific associations detected with some genes, consistent with the possibility that the different psychopathology in these disorders is modulated by some DA genes. Supporting a number of prior reports, we found nominally significant associations between two *DRD2* SNPs and SZ/SZA, but not BP1 (Glatt et al., 2008; Monakhov, Golimbet, Abramova, Kaleda, & Karpov, 2008) (Dubertret et al., 2004). Both associations replicated risk alleles detected by Sanders and colleagues (Sanders et al., 2008). We also detected a trend for association with a SNP that was a surrogate for another associated SNP (rs4245147) in that study (rs4274224, uncorrected p = 0.09; see supplementary Table 6.2). None of these SNPs met significance thresholds in our study or Sanders et al. after corrections for multiple testing were applied. These results highlight the inherent difficulty with interpreting nominally significant results in large scale studies, where sample sizes are rarely sufficient to detect replicable associations of small effect. We did not find an association with two surrogates for rs6277 (C957T) (Hanninen et al., 2006; Hoenicka et al., 2006; Lawford et al., 2005; H. Xu et al., 2007). Glatt et al. also reported associations with SNPs and haplotypes spanning most regions of *DRD2* in an Asian population. While a non-significant trend in that study was consistent with our observed association (rs7131056), the most significant SNPs did not overlap with our results, lending credence to the argument that the primary *DRD2* risk alleles remain unidentified (Glatt et al., 2008). In BP1, we detected association with five *FREQ* SNPs. A number of

other strong BP1 candidate such as *MAOB* and *SLC18A1* (VMAT1) were nominally significant in BP1 but not SZ/SZA (see Table 12).



SNP	Gene region	BP1 p-value	SZ/SZA p-value
rs2046496	3'	**0.017**	**0.034**
rs12636133	3'	**0.009**	**0.024**
rs10934254	3'	**0.009**	**0.037**
rs9868039	3'	**0.002**	**0.004**
rs9817063	3'	**0.003**	**0.012**
rs3732790	3'	**0.012**	**0.033**
rs2134655	IN5	**0.005**	**0.008**
rs963468	IN4	**0.008**	**0.030**
rs324035	IN3	0.873	0.488
rs2630351	IN3	0.115	0.263
rs167771	IN3	0.626	0.459
rs324032	IN3	*0.082*	0.149
rs167770	IN2	0.230	0.232
rs226082	IN2	0.249	0.254
rs324030	IN2	0.249	0.254
rs7625282	IN2	*0.073*	*0.058*
rs10934256	IN2	0.132	**0.035**
rs7633291	IN2	0.147	**0.046**
rs6280	EX2	0.681	0.557
rs1800828	IN1	0.523	0.165

Figure 13 Linkage disequilibrium (r^2) between *DRD3* SNPs and association statistics

Linkage disequilibrium (r^2), SNP locations, and tests of association in both diagnostic groups for all DRD3 SNPs provided. IN = intron, EX = exon. Genomic locations based on dbSNP build 129, which includes an exon 5' to previously described exon 1. P-values based on Armitage trends test. All p-values are uncorrected for multiple comparisons.

Our results support the null hypothesis for several traditional susceptibility candidates. At *COMT* previous studies suggested associations with SZ and multiple SNPs / haplotypes spanning the gene, including our own (H. J. Williams et al., 2007) (Talkowski et al., 2008). In the current study, we tested 31 variations and found no probabilities lower than $p = 0.217$. At *DBH*, we evaluated 29 SNPs with $p > 0.12$ for all tests (refs). *MAOA* was similarly negative in SZ, as well as BP1. As discussed, the power of our study was relatively low to detect small effects, but our study provides compelling evidence against individual SNPs of major effect within these genes.

We previously reported on associations between SZ/SZA at four DA genes (*DRD3, SLC6A3, SLC18A2, COMT*). Nominally significant associations with individual SNPs were detected with three these genes (not *COMT*), an expected result given the overlap between SZ/SZA samples in the present and prior studies (Talkowski et al., 2008). Most of the associated SNPs differed between studies, althought non-significant trends were noted for some alleles (e.g. rs3756450). Still, only the *DRD3* findings provided significant overlap with prior studies, and no results were significant after corrections for multiple testing. Such variation might be expected in view of the different control groups utilized, the possibility of over-inflation of the effect size in the initial study, or stochastic variation if the primary risk alleles are not analyzed.

There are limitations to this study. The associations identified here may not represent primary risk alleles, so analysis of additional common and / or rare variants may be needed. Independent samples, preferably large Caucasian cohorts, are critical to strengthen support for a shared etiology between BP1 and SZ/SZA. Additional studies to investigate the biological function of the associated SNPs are also required. Owing to

limitations imposed by power in the available samples, rare variants or structural variations within DA genes were not investigated. Such efforts are worthy, in view of recent suggestions regarding the role of rare variations in SZ genesis (Walsh et al., 2008; B. Xu et al., 2008).

The present sample was adequately powered to detect associations with odds ratios of 1.5 or greater when individual genes were considered independently, but power to detect associations when all loci were considered was inadequate. In addition, our analytic strategy was conservative and could limit interpretation of these results. Previously investigated DA genes were equally weighted with novel DA interacting genes, raising the possibility of type II errors. For example, *DRD2* had strong a priori evidence as an SZ/SZA susceptibility candidate, and both our study and Sanders et al. (Sanders et al., 2008) identified associations at *DRD2*, however neither set of associations were significant when corrected for all other genes considered. Similarly, the epistatic interactions entailed a considerable number of tests, so our power was limited to detect only very large effect sizes after correcting for multiple tests. None of the interactions results were significant after such corrections.

We previously suggested interactions between four DA genes and SZ/SZA (*SLC6A3*COMT, SLC6A3*DRD3, SLC6A3*SLC18A2,* and *DRD3*SLC18A2*) (Talkowski et al., 2008). When we evaluated those genes here, we found 198 nominally significant interactions for SZ/SZA *vs* control comparisons, which were present in each of gene of the pairs (198 / 1457 tests = 13.59%), consistent with our earlier report using a different set of controls. When BP1 cases were compared with the controls, 188 of these SNP pairs were also significantly associated with risk (12.9%). Despite considerable

effect sizes for some of these analyses (Supplementary Table 6.5), none of these results exceeded chance expectations when all interactions were considered.

In conclusion, we conducted a comprehensive evaluation of the DA pathway for common variants conferring risk for BP1 and SZ/SZA. Our analyses identified *DRD3* as the strongest susceptibility gene for both groups. We found additional support for other overlapping and disease specific risk loci in each disorder (approximately 60% of nominally significant SZ/SZA associations were also associated with BP1). Our analyses suggest the overlapping risk loci could not be entirely attributed to population substructure or variation due to control selection. Other associations were significant for BP1 or SZ/SZA, suggesting modulatory DA influences that could impact differences in psychopathology between these disorders. Our findings could provide a genetic epidemiological basis for a shared etiology between SZ, SZA, and BP1,

6.6 ACKNOWLEDGEMENTS

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7.0 SUMMARY AND CONCLUSIONS

7.1 SUMMARY OF STUDIES

This series of studies was aimed at evaluating a genetic basis for the commonly cited hypothesis that the dopaminergic neurotransmitter pathway is dysregulated in the pathogenesis of schizophrenia. The initial review of the literature suggested a clear pattern in which strong claims and subsequent conclusions have been drawn regarding dopaminergic gene variations despite a paucity of information for most genes. Paralleling the technological advances in molecular genetics that has taken place over a remarkably short period of time, each of the studies presented incrementally grew in terms of molecular and analytic sophistication, culminating with analysis of a considerable portion of representative common variation in the dopaminergic network.

The initial study of *DRD3* addressed a significant gap in the literature regarding this important autoreceptor in the dopaminergic pathway. Prior to 2006, nearly all studies of *DRD3* evaluated only a single coding variant, rs6280 ($Ser^{9}Gly$) in exon 1 (now exon2) of the gene. Our linkage disequilibrium analyses showed that this variant was independent of other regions in the gene, and the vast majority of sequence variation remained uncovered. Our study considered SNPs over a 109 kb region and was the first to suggest associations between schizophrenia and SNPs / haplotypes 3' to rs6280. Our

study was also one of the few to seek evidence of replication as part of the initial investigation. Despite the incremental advances of this study over previous efforts, the analyses shared many of the limitations of its predecessors. Representative variations spanning the gene were not yet publicly available, power was limited, and a large number of tests were conducted without correcting for multiple comparisons. Nonetheless, associations were subsequently replicated in an independent population (Dominguez et al., 2007), indicating more comprehensive analyses were warranted.

Study #2 was motivated by similar logic to study #1. The majority of genes central to the dopaminergic pathway had been previously studied, but usually in small samples with no more than one or two putatively functional variations. This study employed a multi-stage design. The first stage was conducted in a small trio sample just prior to availability of the International HapMap Project (HapMap, 2003), so the initial screen was limited to detecting test statistics falling on the extreme end of the distribution. Still, an internal replication in the US cohort identified several consistent associations. Follow-up analyses using a more comprehensive set of polymorphisms validated the initial screen, and consistencies with a replicate Bulgarian cohort were also encouraging. To our knowledge, this was the first study to report replicated interactions between dopaminergic variations and schizophrenia. Our simulations suggested these findings were unlikely under the null hypothesis. Results at the dopamine transporter were particularly consistent, and functional analyses suggested a novel transcription factor binding sites and effects on promoter activity with an associated SNP 5' to the transcription start site. This combination of statistical and functional associations suggested the dopamine transporter was a promising target for future studies; however

detection of a primary risk allele for the disorder was not obvious as the associations spanned several independent genomic regions.

Study #3 was an effort peripheral to the series of investigations on the neurobiological impact of dopaminergic genes on schizophrenia risk. Phenylalanine hydroxylase catalyzes the hydroxylation of phenylalanine to tyrosine, a dopamine precursor, in the kidney, liver, and melanocytes (Lichter-Konecki, Hipke, & Konecki, 1999; S. C. Richardson & Fisher, 1993; Schallreuter et al., 1995; Solstad, Stokka, Andersen, & Flatmark, 2003). Mutations in PAH combined with phenylalanine in the diet can cause phenylketunuria (PKU), a severe neurological disorder. Co-segregation of PKU with psychiatric illness has been reported (Fisch et al., 1979; Penrose, 1935), and a series of studies suggested association with several PKU causing variants and schizophrenia in small cohorts of Caucasian and African-American subjects (M. A. Richardson et al., 2003). Our study extended these previous results to evaluate both the common and rare variant hypotheses of schizophrenia in four independent samples of Caucasian and African-American ancestry. One common *PAH* SNP was associated with schizophrenia in two independent samples (rs1522305). The association was with an intronic variant, and functional analyses are necessary in future studies.

Study #4, was a natural endpoint to these surveys of common dopaminergic polymorphisms and their role in schizophrenia etiology. This study addressed the limitations in coverage of studies #1 and #2, as well as the narrow view of the dopamienrgic network considered in study #2. Motivated by an extensive literature suggesting shared etiological risk factors for schizophrenia, schizoaffective disorder, and bipolar disorder, this study simultaneously evaluated SNPs reporesentative of all publicly

available common variation within 40 genes and compared these disorders to a common set of screened adult controls. The results provided strong evidence for *DRD3* as a shared genetic risk locus for both schizophrenia and bipolar disorder. Since case samples overlapped between studies #2 and 4, the results are not an independent replication. However the findings bolster the argument that *DRD3* is a credible risk locus since each of the three studies in this series evaluating *DRD3* detected significant associations despite comparisons with independent control subjects in each study. The results pertaining to bipolar disorder are novel. The overlap in associations included a cluster of 8 SNPs spanning intron 4 to the 3' region of the gene. Replications of previous findings at other genes were noted. None of the results were significant after corrections for multiple comparisons were applied. Although additional associations were observed at our at our three other primary schizophrenia targets (*SLC18A2*, *SLC6A3*, and *COMT*), none of these results exceeded chance expectations, a result that should raise caution in interpretation of study #2 given the overlap in case samples between studies. In summary, these findings support a shared etiological basis for schizophrenia and bipolar disorder at *DRD3* and several other targets. Although additional common variations are not required in future studies of Caucasians, replication of these findings in additional samples is essential. The absence of replication precludes firm conclusions regarding the nominally significant associations and interactions detected.

7.2 LIMITATIONS

Genetic association studies in psychiatric genetics have had considerable limitations, many of which hindered more conclusive interpretation of the studies conducted herein. Primary among these is the heterogeneous phenotype and individual risk variants of presumably very small effect. Owing to the combination of these two factors, the two primary goals of these studies (comprehensive evaluation and statistically conservative interpretation) may be fundamentally unrealistic in sample sizes such as these. This issue has been widely debated in the literature regarding genomewide association studies. The primary limitation is power. As detailed above, these sampled were reasonably powered to detect an odds ratio of 1.5 under ideal circumstances (including genotyping the actual risk allele). This effect size is almost certainly inflated based on credible large scale studies, and considers only an individual test (i.e. an alpha of 0.05). If one were to consider detection of a true association under the realistic circumstances of these studies (maximum OR of 1.31, $r^2 < 0.9$ between risk allele and surrogate marker, 422 SNPs / disorder, 339 effective tests), over 8,000 cases would be required to detect statistical significance after correction. The threshold for significance is more stringent in a GWAS study, a factor likely contributing to the lack of consistency between such psychiatric genetic studies to date. Statistical weighting may be an optimal strategy, but the nebulous nature of determining what constitutes a significant association in the existing literature complicates such designs.

Another limitation was the inability to draw conclusions regarding primary risk causing variants. The functional analyses in study #2 were intriguing but not conclusive. The validity of an *in vitro* assay system to model *in vivo* effects in the brain is always a

concern. The majority of the associations detected were with intronic variations of yet unknown function. Finally, these results raise the question of statistical thresholds for interpreting replication between independent samples. These studies relied on strategies such as joint analyses and summary statistics to identify multiple independent small effects in the same direction (i.e. the same risk allele). This strategy is defensible given the power limitations discussed above, but could be interpreted as anti-conservative.

7.3 ONGOING AND FUTURE STUDIES

These studies represent a reasonable evaluation of the common variant hypothesis of genetic risk to schizophrenia (and bipolar disorder). If the common variant hypothesis were to be evaluated further, comprehensive sequencing for novel SNP detection might provide the most benefit in identifying true liability loci. Our ongoing study has taken this approach to exhaustively evaluate the dopamine transporter. In conjunction with the SeattleSNPs project, we have conducted focused sequencing to cover roughly 90% of the genomic region spanning the gene, cataloguing all common and rare variations detected. To date, 375 variations have been catalogued, 164 of which are common. We have genotyped 88 tag SNPs in both the US and Bulgarian samples. We find that consistent associations persist between samples within introns 3 and 4 of the gene. In collaboration with Dr. Javier Lopez at Carnegie Mellon University, novel splice variants are being investigated in this region to determine a plausible functional basis for the associations.

Replicate studies on substantially powered samples are critically needed to validate the associations reported here. These studies would be particularly useful in

interpretation of the most significant interactions detected in study #4. Alternative strategies such as multifactor dimensionality reduction may be of benefit provided replicate samples are available. Additional common variants would be required for individuals of African descent, but replicate studies in Caucasian and Asian populations would likely choose to focus on the subset of noteworthy SNPs already detected (e.g. the top 10% of SNP associations). Additional studies of diagnostic sub-phenotypes would also be of interest given the results of exploratory analyses in study #4. Parallel studies to identify causative rare and structural variations are also desirable. Finally, functional analyses of patient specific cell lines, post-mortem brain tissue, and neuroimaging variables could all yield further lines of inquiry into the genetic epidemiological results reported here.

7.4 CONCLUSION

Taken together, thes analyses failed to conclusively support or reject the null hypothesis of no association between dopaminergic variation and schizophrenia. No obvious individual risk loci arose. The statistical results are compelling that an individual common variant of significant effect on schizophrenia risk (odds ratio greater than 1.5) is unlikely likely to be present within the dopmaniergic network studied here. Instead, a series of small but consistent effects in several genes, as well as replicable epistatic interactions, suggest a plausible genetic basis for the dopamine hypothesis of schizophrenia could exist. The results of these studies require further replication before an alternative hypothesis can be confidently accepted.

APPENDEX A: SUPPLEMENT TO CHAPTER 4

Supplementary Table numbers include chapter and table (e.g. 4.1 = supplementary table 1 from chapter 4).

Table 14 Supplementary Table 4.1: All SNP association tests

					US samples (478 cases / 501 controls)				Bulgaria (659 trios)			Joint
Gene	BP	SNP	Group	N	Case Freq	Cord Freq	p-value	Z_1	Freq	Z_2	P_2	Z_{joint}
DRD3	115313209	rs7631540	ALL	C	0.517	0.520	0.884	-0.14	0.547	-0.80	0.422	-0.70
DRD3	115317621	rs2046496	ALL	C	0.507	0.505	0.916	0.10	0.526	-1.04	0.300	-0.71
DRD3	115319654	MT_4*	ALL	A	0.949	0.954	0.572	-0.56	0.96	0.10	0.922	-0.29
DRD3	115322414	rs12636133	ALL	G	0.571	0.564	0.747	0.32	0.549	0.16	0.872	-0.09
DRD3	115324324	rs10934254	ALL	A	0.570	0.562	0.694	0.39	0.547	0.41	0.685	0.56
DRD3	115329232	rs9868039	ALL	C	0.558	0.550	0.572	0.33	0.572	-0.38	0.707	-0.07
DRD3	115329798	rs9817063	ALL	A	0.538	0.547	0.686	-0.41	0.524	0.20	0.839	-0.11
DRD3	115329973	rs3732790	ALL	T	0.608	0.625	0.438	-0.77	0.599	0.58	0.564	-0.07
DRD3	115337545	rs13061336	ALL	A	0.996	0.997	0.160	-1.41	0.997	1.27	0.206	0.03
DRD3	115340891	rs2134655	ALL	G	0.727	0.762	0.066	-1.88	0.74	0.18	0.857	1.32
DRD3	115345577	rs963468	US	G	0.612	0.628	0.462	-0.74				
DRD3	115352768	rs3773678	ALL	C	0.845	0.848	0.690	-0.60	0.851	0.85	0.393	0.52
DRD3	115358965	rs167771	ALL	T	0.818	0.801	0.348	0.94	0.801	-0.54	0.589	0.20
DRD3	115364131	rs324030	ALL	C	0.724	0.675	0.016	2.40	0.688	0.75	0.455	2.14
DRD3	115364217	rs7625282	ALL	T	0.760	0.710	0.039	2.51	0.744	0.64	0.524	2.13
DRD3	115367825	rs11706283	BGT	C					0.883	-0.74	0.459	
DRD3	115368342	rs10934256	ALL	G	0.820	0.775	0.014	2.46	0.8	0.68	0.494	-1.10
DRD3	115373505	rs6280	ALL	A	0.680	0.627	0.012	2.48	0.667	0.04	0.966	0.08
DRD3	115374239	rs1800828	ALL	G	0.775	0.729	0.015	2.39	0.776	-0.48	0.630	1.20
SLC6A3	1436550	rs27074	ALL	G	0.908	0.905	0.829	0.21	0.912	0.28	0.782	0.07
SLC6A3	1444369	rs12516948	ALL	T	0.619	0.673	0.015	-2.47	0.65	-1.27	0.205	-2.58
SLC6A3	1447522	rs27072	ALL	G	0.834	0.808	0.133	1.50	0.82	0.78	0.436	-0.40
SLC6A3	1447815	rs1042098	BGT	T					0.687	0.68	0.500	
SLC6A3	1448077	rs40184	ALL	C	0.530	0.507	0.289	1.04	0.505	0.00	1.000	0.68
SLC6A3	1457548	rs6869645	ALL	C	0.926	0.936	0.392	-0.87	0.922	0.40	0.686	0.88
SLC6A3	1464412	rs6347	ALL	A	0.732	0.710	0.257	1.12	0.745	1.67	0.095	1.99
SLC6A3	1465645	rs27048	ALL	G	0.582	0.582	0.991	-0.01	0.536	0.86	0.392	0.64
SLC6A3	1468629	rs37022	ALL	A	0.833	0.801	0.073	1.81	0.842	0.93	0.354	1.89

Table 14 Continued

SLC6A3	1469646	rs2042449	US	G	0.777	0.763	0.438	0.78				
SLC6A3	1476905	rs464049	ALL	A	0.579	0.524	0.013	2.46	0.53	2.55	0.011	3.54
SLC6A3	1483515	rs456082	ALL	A	0.806	0.766	0.027	2.16	0.771	1.70	0.090	2.70
SLC6A3	1484164	rs463379	ALL	C	0.805	0.767	0.035	2.07	0.772	1.82	0.069	2.73
SLC6A3	1491354	rs403636	ALL	G	0.815	0.848	0.045	-1.98	0.846	-1.45	0.146	-2.39
SLC6A3	1495521	rs2617605	ALL	HWE Flag_removed					0.685	-0.45	0.655	-0.98
SLC6A3	1496199	rs6350	US	G	0.937	0.932	0.636	0.47				
SLC6A3	1501148	rs3756450	ALL	A	0.893	0.868	0.080	1.70	0.848	2.12	0.035	2.70
SLC6A3	1503506	rs2550947	ALL	C	0.584	0.572	0.570	0.56	0.559	1.07	0.286	0.44
SLC6A3	1505280	rs2078247	ALL	G	0.721	0.763	0.030	-2.16	0.769	-0.15	0.882	-1.53
SLC18A2	118995757	rs363393	ALL	A	0.812	0.793	0.315	1.09	0.839	1.89	0.059	2.14
SLC18A2	118998861	rs363399	ALL	T	0.769	0.749	0.319	1.02	0.773	-0.37	0.713	0.39
SLC18A2	118999379	rs363338	ALL	T	0.707	0.660	0.029	2.23	0.666	-0.74	0.458	2.02
SLC18A2	119004938	rs363343	ALL	A	0.824	0.786	0.046	2.09	0.799	-0.28	0.782	1.16
SLC18A2	119008669	rs2283138	ALL	A	0.892	0.895	0.839	-0.21	0.887	-0.48	0.635	-0.49
SLC18A2	119009116	rs929493	ALL	T	0.840	0.808	0.068	1.86	0.815	-0.06	0.956	1.18
SLC18A2	119009680	rs4752045	ALL	G	0.603	0.542	0.009	2.72	0.515	0.43	0.665	-1.46
SLC18A2	119011397	rs10082463	ALL	A	0.909	0.894	0.260	1.15	0.894	-0.28	0.784	0.54
SLC18A2	119012563	rs363224	ALL	A	0.551	0.528	0.341	1.03	0.512	1.62	0.106	1.90
SLC18A2	119015202	rs363226	ALL	C	0.668	0.646	0.324	1.03	0.608	0.70	0.486	1.20
SLC18A2	119016556	rs363227	ALL	C	0.891	0.868	0.184	1.40	0.87	1.51	0.130	2.02
SLC18A2	119027061	rs14240	US	C	0.536	0.523	0.702	0.15				
SLC18A2	119028361	rs363236	ALL	T	0.859	0.843	0.352	0.98	0.835	-0.57	0.570	0.21
SLC18A2	119029149	rs363285	ALL	A	0.712	0.718	0.800	-0.27	0.678	1.25	0.210	0.77
COMT	18303438	rs2020917	ALL	C	0.684	0.712	0.160	-1.18	0.673	1.04	0.296	1.56
COMT	18305961	rs933271	ALL	A	0.723	0.723	0.972	0.04	0.775	1.58	0.113	1.22
COMT	18310121	rs737865	ALL	A	0.684	0.712	0.163	-1.39	0.679	0.88	0.379	-0.80
COMT	18319731	rs740603	ALL	C	0.603	0.584	0.317	0.84	0.547	0.59	0.552	-0.10
COMT	18322680	rs7290221	BGT	G					0.53	0.75	0.456	
COMT	18322891	rs4646312	ALL	A	0.584	0.613	0.185	-1.31	0.6	-0.45	0.654	-1.19
COMT	18323417	rs165656	ALL	C	0.515	0.504	0.634	0.47	0.512	1.48	0.140	1.43
COMT	18324506	rs6269	US	T	0.581	0.609	0.198	-1.29				
COMT	18324982	rs2239393	US	T	0.582	0.609	0.212	-1.25				
COMT	18325825	rs4680	US	A	0.510	0.500	0.642	0.47				
COMT	18326451	rs4646315	ALL	G	0.831	0.828	0.898	0.13	0.836	-1.38	0.169	-0.95
COMT	18326686	rs4646316	ALL	C	0.756	0.755	0.967	0.04	0.777	0.29	0.775	0.25
COMT	18327115	rs165774	ALL	C	0.682	0.689	0.738	-0.33	0.667	-0.79	0.431	-0.81
COMT	18327730	rs174696	ALL	T	0.794	0.757	0.048	1.99	0.782	1.43	0.154	2.38
COMT	18330235	rs4633	BGT	C					0.501	-0.04	0.965	
COMT	18330246	rs9332377	ALL	C	0.836	0.858	0.172	-1.32	0.835	-0.41	0.685	-1.17
COMT	18330763	rs740601	BGT	A					0.584	-0.99	0.323	
COMT	18331107	rs9332381	ALL	G	0.956	0.954	0.833	0.21	0.986	0.23	0.819	0.31
COMT	18331207	rs4818	BGT	C					0.588	-0.88	0.380	
COMT	18331335	rs165599	US	T	0.689	0.688	0.943	0.07				
COMT	18333223	rs165849	US	A	0.688	0.687	0.936	0.08				
COMT	18334027	rs165815	ALL	T	0.862	0.833	0.076	1.81	0.84	1.31	0.189	2.18

*Table 14. BP = genomic location in base pairs. N = nucleotide of common allele. Freq = frequency of common allele provided. Z scores are test statistics with reference to common allele. allele. p-value = p-value obatined from Armitage Trends test comparing case and control genotype distributions. BGT = Bulgarian trios. Frequency of common allele for parents of probands in Bulgarian families provided. Joint = joint analysis. Z_{joint} derived from the joint distribution of test statistics from stages II and III, weighted for sample size. *Novel SNP discovered from sequencing.*

APPENDIX B: SUPPLEMENT TO CHAPTER 5

Table 15 Supplementary Table 5.1: Nominally significant gender based analyses

		Associations in Females only				Associations in Males only			
SNP	Gene Location	Bulgarian p-value	US C-C p-value	US Trio p-value	AFAM p-value	Bulgarian p-value	US C-C p-value	US Trio p-value	AFAM p-value
rs2245360	Exon-11					0.02			
rs1042503	***Exon-7***					***0.05***		***0.01***	
rs1126758	***Exon-6***					***0.02***		***0.07***	
rs12425434	***Intron-5***	0.03				***0.07***		***0.004***	
rs937476	Intron-6					0.004			
rs2037639	Intron-3					***0.03***		***0.03***	
rs1722392	Intron-3					0.04			
rs1522305	***Intron-3***	***0.002***	***0.05***				0.04		
rs1522296	Intron- 1	0.007							

Nominally significant SNP tests for gender based analyses. P-values provided for unconditional (case-control) or conditional (family based) logistic regression among male or female participants separately.. All p-values are uncorrected for multiple comparisons. C-C = case-control. AFAM = African-American families. SNPs where nominally significant replication ($p < 0.05$) was detected are highlighted. No SNPs were significant after correction.

APPENDIX C: SUPPLEMENT TO CHAPTER 6

Table 16 Supplementary Table 6.1: All gene descriptions

					SNP Analyses (Trends Test)		Gene-based test (Hotelling's T^2)	
Chr	**Gene**	**Name**	**Tag SNPs**	**Effective tests**	**Best SZ p-value**	**Best BD p-value**	**Empirical p-value (SZ)**	**Empirical p-value (BP1)**
22	ADRBK2	adrenergic, beta, receptor kinase 2	3	1.0	0.516	0.269	0.756	0.700
22	CACNG2	calcium channel, voltage-dependent, gamma subunit 2	6	2.7	0.063	0.175	0.247	0.374
21	CLIC6	chloride intracellular channel 6	6	2.4	0.280	0.2085	0.881	0.734
22	COMT	catechol-O-methyltransferase	31	10.6	0.217	0.050	0.804	0.518
7	COPG2	Gamma COP	6	2.7	0.408	0.260	0.801	0.615
17	DARPP32	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1	1.0	0.144	0.855	0.179	0.849
9	DBH	dopamine beta-hydroxylase	29	11.2	0.124	0.045	0.617	0.878
7	DDC	dopa decarboxylase	40	**11.9**	0.029	0.002*	0.391	0.708
5	DRD1	dopamine receptor 1	5	1.8	0.095	0.536	0.400	0.988
10	DRD1IP	dopamine receptor D1 interacting protein	4	1.9	0.012*	0.008*	0.090	0.063
11	DRD2	dopamine receptor D2	22	8.3	0.037	0.261	0.433	0.813

Table 16 Continued

3	DRD3	dopamine receptor D3	20	6.7	0.003*	0.002*	0.007	0.013
11	DRD4	dopamine receptor D4	2	1.0	0.613	0.052	0.745	0.095
4	DRD5	dopamine receptor D5	1	1.0	0.646	0.600	0.597	0.577
12	DRIP78	dopamine receptor interacting protein	3	1.0	0.428	0.978	0.496	1.000
1	EPB41	erythrocyte membrane protein band 4.1	15	4.9	0.138	0.022	0.818	0.109
X	FLNA	Filamin A	2	1.0	0.396	0.340	0.060	0.208
9	FREQ	frequenin homolog	21	8.1	0.056	0.011	0.776	0.376
5	GNB2L1	Receptor for activated C kinase1	4	1.9	0.234	0.245	0.150	0.419
17	GRB2	growth factor receptor-bound protein 2	8	3.4	0.037	0.208	0.373	0.715
11	GRK2	adrenergic, beta, receptor kinase 1	2	1.0	0.467	0.223	0.671	0.447
8	Hey1	Hesr1/Hey1	7	2.6	0.530	0.083	0.992	0.156
16	HIC5	Focal adhesion protein	2	1.0	0.657	0.154	0.828	0.360
X	MAOA	monoamine oxidase A	8	3.1	0.228	0.318	0.336	0.481
X	MAOB	monoamine oxidase B	8	3.3	0.019	0.042	0.268	0.296
3	NCK1	NCK adaptor protein	6	2.6	0.316	0.565	0.826	0.977
8	NEF3	Neurofilament M	8	3.5	0.044	0.075	0.342	0.286
2	NR4A2	nuclear receptor subfamily 4, group A, member 2	2	1.0	0.384	0.431	0.682	0.643
22	PICK1	Protein interacting with C Kinase1	6	2.6	0.150	0.319	0.697	0.931
17	PPP1R9B	Spinophilin	5	1.9	0.068	0.019*	0.396	0.095
5	PPP2CA	Protein Phosphatase	1	1.0	0.486	0.994	0.566	1.000
8	SLC18A1	solute carrier family 18 (vesicular monoamine), member 1	22	8.7	0.233	0.005*	0.761	0.113
10	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	18	7.7	0.041	0.095	0.420	0.393
5	SLC6A3	Dopamine Transporter	47	16.9	0.045	0.118	0.479	0.818

20	SNAP25	synaptosomal-associated protein	34	13.3	0.064	0.012	0.334	0.375
4	SNCA	Synuclein	12	4.9	0.128	0.038	0.753	0.614
7	Sp4	Sp4 transcription factor	3	1.1	0.016*	0.687	0.105	0.963
7	STX1A	Syntaxin1A	2	1.0	0.455	0.771	0.572	0.956
16	SYNGR3	synaptogyrin 3	2	1.0	0.468	0.662	0.650	0.885
11	TH	tyrosine hydroxylase	7	2.7	0.098	0.106	0.247	0.252

**Significant after gene-wide correction*.

Table 17 Supplementary Table 6.2: All SNP associations in both diagnostic groups

Genomic Information			Minor Allele Information				Test Statistics			
Gene	Position	SNP	Nuc	SZ/SZA Freq	BP1 Freq	Control Freq	SZ/SZA p-value	SZ OR	BP1 p-value	BP1 OR
EPB41	29092857	rs126013	A	0.355	0.347	0.376	0.3185	0.91	0.1734	0.88
EPB41	29101418	rs150093	G	0.138	0.142	0.141	0.8521	0.98	0.9473	1.01
EPB41	29114784	rs203278	G	0.333	0.336	0.363	0.1723	0.88	0.2036	0.89
EPB41	29117993	rs157208	G	0.217	0.231	0.220	0.8791	0.98	0.5486	1.07
EPB41	29131901	rs150089	G	0.303	0.305	0.324	0.3223	0.91	0.3623	0.92
EPB41	29141303	rs12038347	G	0.088	0.105	0.091	0.8187	0.96	0.2951	1.17
EPB41	29182405	rs2762682	A	0.286	0.281	0.278	0.6996	1.04	0.8698	1.02
EPB41	29183661	rs12021667	G	0.409	0.412	0.397	0.6053	1.05	0.5015	1.06
EPB41	29205312	rs11581096	A	0.097	0.094	0.108	0.4145	0.89	0.2889	0.85
EPB41	29207950	rs2985331	G	0.142	0.141	0.132	0.5040	1.09	0.5820	1.08
EPB41	29229250	rs10915216	A	0.498	0.483	0.491	0.7438	1.03	0.2339	0.90
EPB41	29269534	rs12130351	A	0.060	0.049	0.052	0.4781	1.15	0.7094	0.93
EPB41	29316310	rs2249138	A	0.121	0.109	0.133	0.4013	0.89	*0.0977*	0.80
EPB41	29317814	rs12120422	G	0.168	0.164	0.165	0.8406	1.03	0.9476	0.99
EPB41	29319434	rs575675	G	0.086	0.075	0.104	0.1825	0.82	**0.0216**	0.70
NR4A2	156890171	rs12803	A	0.474	0.476	0.494	0.3793	0.93	0.4312	0.93
NR4A2	156891881	rs834834	G	0.308	0.316	0.321	0.5524	0.94	0.8362	0.98
DRD3	115317621	rs2046496	G	0.494	0.502	0.448	**0.0345**	1.21	**0.0151**	1.24
DRD3	115322414	rs12636133	C	0.424	0.416	0.475	**0.0239**	0.81	**0.0080**	0.79
DRD3	115324324	rs10934254	G	0.428	0.417	0.475	**0.0367**	0.83	**0.0091**	0.79
DRD3	115329232	rs9868039	A	0.451	0.458	0.387	**0.0039**	1.30	**0.0013**	1.34
DRD3	115329798	rs9817063	G	0.456	0.447	0.514	**0.0123**	0.79	**0.0031**	0.77
DRD3	115329973	rs3732790	A	0.383	0.374	0.431	**0.0334**	0.82	**0.0094**	0.79
DRD3	115340891	rs2134655	A	0.277	0.280	0.226	**0.0080**	1.31	**0.0059**	1.33
DRD3	115345577	rs963468	A	0.381	0.370	0.429	**0.0302**	0.82	**0.0074**	0.78
DRD3	115351544	rs324035	A	0.210	0.200	0.197	0.4879	1.08	0.8611	1.02
DRD3	115357749	rs2630351	A	0.065	0.070	0.053	0.2630	1.24	0.1304	1.33
DRD3	115358965	rs167771	G	0.187	0.183	0.174	0.4593	1.09	0.6161	1.06
DRD3	115360518	rs324032	G	0.069	0.072	0.053	0.1492	1.32	*0.0935*	1.37
DRD3	115362252	rs167770	G	0.277	0.277	0.301	0.2323	0.89	0.2448	0.89
DRD3	115363703	rs226082	G	0.278	0.278	0.301	0.2537	0.89	0.2647	0.90
DRD3	115364131	rs324030	G	0.278	0.278	0.301	0.2537	0.89	0.2647	0.90
DRD3	115364217	rs7625282	G	0.248	0.250	0.284	*0.0575*	0.83	*0.0808*	0.84
DRD3	115368342	rs10934256	A	0.178	0.188	0.215	**0.0355**	0.79	0.1389	0.85
DRD3	115369758	rs7633291	C	0.180	0.189	0.215	**0.0463**	0.80	0.1541	0.85
DRD3	115373505	rs6280	G	0.325	0.329	0.338	0.5569	0.95	0.6817	0.96
DRD3	115374239	rs1800828	G	0.226	0.239	0.252	0.1651	0.87	0.5140	0.93
NCK1	138069807	rs9845460	A	0.222	0.214	0.206	0.3733	1.10	0.6333	1.05
NCK1	138101599	rs9867325	C	0.235	0.229	0.237	0.9338	0.99	0.6938	0.96
NCK1	138102256	rs6783508	A	0.317	0.298	0.296	0.3055	1.11	0.8957	1.01
NCK1	138109387	rs1347209	C	0.397	0.380	0.380	0.4242	1.08	0.9968	1.00
NCK1	138150095	rs1048145	G	0.095	0.084	0.089	0.6538	1.07	0.6930	0.94
NCK1	138154451	rs7648198	C	0.453	0.442	0.441	0.6065	1.05	0.9863	1.00
DRD5	9397033	rs2867383	A	0.323	0.324	0.312	0.5966	1.05	0.5614	1.06
SNCA	90862167	rs168552	G	0.280	0.286	0.253	0.1618	1.15	*0.0945*	1.18
SNCA	90865909	rs356165	G	0.383	0.369	0.351	0.1284	1.15	0.3935	1.08
SNCA	90910560	rs356188	G	0.221	0.238	0.203	0.3337	1.11	*0.0620*	1.22

Table 17 Continued

SNCA	90912499	rs356164	C	0.132	0.146	0.128	0.7939	1.04	0.2330	1.17
SNCA	90924387	rs356186	A	0.206	0.225	0.195	0.5402	1.07	0.1037	1.20
SNCA	90931534	rs10002435	A	0.121	0.108	0.120	0.9454	1.01	0.4194	0.89
SNCA	90950732	rs1866995	G	0.058	0.048	0.055	0.7385	1.07	0.4959	0.87
SNCA	90955540	rs3822095	G	0.377	0.373	0.402	0.2378	0.90	0.1893	0.89
SNCA	90959901	rs2737020	G	0.285	0.298	0.269	0.4449	1.08	0.1527	1.15
SNCA	90964953	rs6532191	G	0.484	0.503	0.457	0.2136	1.11	**0.0404**	1.20
SNCA	90976758	rs2619361	A	0.262	0.266	0.252	0.5999	1.06	0.4690	1.08
SNCA	90980380	rs17016274	A	0.067	0.061	0.069	0.8587	0.97	0.4611	0.87
SLC6A3	1436550	rs27074	A	0.091	0.081	0.087	0.7472	1.05	0.6439	0.93
SLC6A3	1443349	rs12516758	G	0.224	0.207	0.207	0.3495	1.11	0.9999	1.00
SLC6A3	1444161	rs11133762	A	0.014	0.023	0.019	0.4105	0.75	0.5254	1.22
SLC6A3	1445711	rs3863145	A	0.262	0.274	0.272	0.6318	0.95	0.8848	1.02
SLC6A3	1447522	rs27072	A	0.160	0.166	0.165	0.7663	0.96	0.9315	1.01
SLC6A3	1447815	rs1042098	G	0.272	0.276	0.275	0.9088	0.99	0.9340	1.01
SLC6A3	1448077	rs40184	A	0.458	0.431	0.457	0.9569	1.01	0.2483	0.90
SLC6A3	1451007	rs11564772	A	0.081	0.071	0.082	0.9278	0.99	0.3532	0.86
SLC6A3	1452431	rs11564769	G	0.083	0.069	0.080	0.8368	1.04	0.3541	0.85
SLC6A3	1457548	rs6869645	A	0.067	0.056	0.066	0.9058	1.02	0.3687	0.85
SLC6A3	1457704	rs11564767	T	0.079	0.065	0.068	0.3653	1.17	0.7794	0.95
SLC6A3	1458694	rs28363119	G	0.065	0.056	0.066	0.9590	0.99	0.3687	0.85
SLC6A3	1458806	rs11564764	A	0.066	0.054	0.064	0.8238	1.04	0.3704	0.84
SLC6A3	1459036	rs6876225	A	0.067	0.056	0.065	0.8317	1.04	0.4212	0.86
SLC6A3	1460129	rs3776511	A	0.205	0.196	0.206	0.9753	1.00	0.5812	0.94
SLC6A3	1461979	rs11133770	C	0.215	0.203	0.211	0.7990	1.03	0.6583	0.95
SLC6A3	1463472	rs2617577	G	0.286	0.266	0.277	0.6577	1.05	0.5999	0.95
SLC6A3	1463613	rs11564762	A	0.207	0.197	0.208	0.9684	1.00	0.5551	0.94
SLC6A3	1464256	rs2550936	C	0.289	0.267	0.281	0.6770	1.04	0.4770	0.93
SLC6A3	1464412	rs6347	G	0.270	0.270	0.274	0.8700	0.98	0.8413	0.98
SLC6A3	1468629	rs37022	A	0.164	0.178	0.174	0.5669	0.93	0.8229	1.03
SLC6A3	1469142	rs40358	C	0.175	0.151	0.147	*0.0868*	1.23	0.7873	1.04
SLC6A3	1469646	rs2042449	A	0.227	0.240	0.224	0.8971	1.01	0.4081	1.09
SLC6A3	1472932	rs2975292	G	0.370	0.361	0.351	0.3687	1.09	0.6378	1.05
SLC6A3	1473268	rs2735917	A	0.057	0.055	0.056	0.8949	1.03	0.9917	1.00
SLC6A3	1473346	rs28382247	A	0.231	0.237	0.231	0.9976	1.00	0.7322	1.04
SLC6A3	1473476	rs28382245	A	0.390	0.391	0.408	0.4112	0.93	0.4450	0.93
SLC6A3	1473588	rs11564758	G	0.392	0.390	0.414	0.3073	0.91	0.2744	0.91
SLC6A3	1476905	rs464049	G	0.423	0.449	0.431	0.7293	0.97	0.4049	1.08
SLC6A3	1483244	rs464061	A	0.193	0.212	0.200	0.6684	0.95	0.5069	1.08
SLC6A3	1483616	rs11737901	A	0.336	0.348	0.362	0.2202	0.89	0.5241	0.94
SLC6A3	1484164	rs463379	C	0.194	0.212	0.200	0.7218	0.96	0.5265	1.07
SLC6A3	1489408	rs420422	G	0.436	0.450	0.438	0.9379	0.99	0.5855	1.05
SLC6A3	1491354	rs403636	A	0.181	0.158	0.153	*0.0924*	1.23	0.7719	1.04
SLC6A3	1495732	rs2981359	C	0.432	0.445	0.471	*0.0764*	0.85	0.2419	0.90
SLC6A3	1495842	rs13189021	A	0.200	0.226	0.237	**0.0487**	0.81	0.5501	0.94
SLC6A3	1495974	rs2254408	C	0.441	0.440	0.456	0.4952	0.94	0.4573	0.94
SLC6A3	1496498	rs2455391	A	0.269	0.233	0.262	0.7415	1.03	0.1360	0.86
SLC6A3	1496728	rs2937639	A	0.414	0.415	0.405	0.6764	1.04	0.6399	1.04
SLC6A3	1497427	rs2963238	A	0.406	0.405	0.398	0.7153	1.03	0.7487	1.03
SLC6A3	1498616	rs2975226	T	0.413	0.410	0.408	0.8086	1.02	0.9071	1.01
SLC6A3	1499389	rs2652511	A	0.405	0.407	0.401	0.8557	1.02	0.7847	1.03

Table 17 Continued

SLC6A3	1501148	rs3756450	G	0.107	0.128	0.131	*0.0986*	0.80	0.8318	0.97
SLC6A3	1505280	rs2078247	G	0.279	0.242	0.268	0.5861	1.06	0.1827	0.87
PPP2CA	133593991	rs4246019	G	0.115	0.124	0.124	0.5266	0.92	0.9679	1.01
DRD1	174800505	rs4867798	G	0.286	0.317	0.321	*0.0890*	0.85	0.8723	0.98
DRD1	174801306	rs686	G	0.373	0.382	0.371	0.9342	1.01	0.6012	1.05
DRD1	174802802	rs5326	A	0.140	0.153	0.158	0.2380	0.86	0.7431	0.96
DRD1	174811672	rs267416	G	0.425	0.406	0.420	0.8341	1.02	0.5246	0.94
DRD1	174813251	rs267418	C	0.463	0.440	0.450	0.5550	1.06	0.6558	0.96
GNB2L1	180595641	rs2261114	G	0.384	0.383	0.408	0.2880	0.91	0.2651	0.90
GNB2L1	180598539	rs13160776	A	0.076	0.074	0.079	0.8148	0.96	0.6733	0.93
GNB2L1	180598882	rs2287716	G	0.133	0.139	0.152	0.2111	0.85	0.3964	0.90
GNB2L1	180602857	rs1279738	C	0.265	0.261	0.262	0.8773	1.02	0.9753	1.00
Sp4	21429057	rs10245440	A	0.234	0.238	0.245	0.5646	0.94	0.7058	0.96
Sp4	21498416	rs12668354	C	0.328	0.278	0.282	**0.0239**	1.24	0.8513	0.98
Sp4	21516416	rs1018954	A	0.429	0.455	0.448	0.4068	0.93	0.7371	1.03
DDC	50492617	rs11575564	A	0.050	0.036	0.034	*0.0602*	1.53	0.7349	1.09
DDC	50492914	rs4947510	A	0.277	0.274	0.298	0.2989	0.90	0.2489	0.89
DDC	50493713	rs11575553	A	0.076	0.093	0.075	0.9700	1.01	0.1656	1.25
DDC	50497803	rs11575548	A	0.094	0.115	0.087	0.5770	1.09	**0.0403**	1.36
DDC	50498481	rs11575542	A	0.025	0.015	0.019	0.3810	1.32	0.5371	0.81
DDC	50499175	rs4947535	A	0.296	0.295	0.340	**0.0339**	0.82	**0.0337**	0.82
DDC	50499488	rs11575535	A	0.033	0.020	0.024	0.2255	1.39	0.5392	0.83
DDC	50503248	rs730092	G	0.416	0.425	0.449	0.1248	0.87	0.2764	0.91
DDC	50504704	rs11575500	A	0.069	0.083	0.061	0.4383	1.15	*0.0539*	1.40
DDC	50511449	rs745043	A	0.206	0.195	0.248	**0.0243**	0.78	**0.0043**	0.73
DDC	50511808	rs4490786	A	0.184	0.213	0.181	0.8721	1.02	*0.0742*	1.22
DDC	50515359	rs11575453	A	0.089	0.097	0.093	0.7460	0.95	0.8035	1.04
DDC	50520392	rs11575441	T	0.013	0.010	0.012	0.7219	1.15	0.6659	0.83
DDC	50520545	rs1451371	G	0.454	0.457	0.428	0.2400	1.11	0.1876	1.13
DDC	50520718	rs11575438	A	0.089	0.097	0.093	0.7460	0.95	0.8035	1.04
DDC	50521365	rs1451372	G	0.392	0.410	0.432	*0.0622*	0.85	0.3254	0.91
DDC	50530192	rs4470989	A	0.297	0.297	0.343	**0.0275**	0.81	**0.0294**	0.81
DDC	50530315	rs4602840	A	0.096	0.117	0.090	0.6406	1.07	*0.0526*	1.33
DDC	50531681	rs6957607	G	0.083	0.088	0.085	0.8475	0.97	0.8086	1.04
DDC	50533884	rs3807563	A	0.410	0.425	0.405	0.8091	1.02	0.3679	1.09
DDC	50534165	rs3807562	A	0.476	0.469	0.446	0.1768	1.13	0.3032	1.10
DDC	50534929	rs11575387	C	0.081	0.084	0.084	0.7925	0.96	0.9825	1.00
DDC	50538516	rs3807558	A	0.207	0.198	0.246	**0.0365**	0.80	**0.0091**	0.75
DDC	50539273	rs11575375	A	0.338	0.347	0.324	0.5059	1.07	0.2739	1.11
DDC	50540203	rs4947584	A	0.340	0.351	0.325	0.4817	1.07	0.2213	1.12
DDC	50540384	rs6592961	A	0.201	0.217	0.193	0.6377	1.05	0.1820	1.16
DDC	50562006	rs10274275	G	0.238	0.253	0.225	0.4946	1.07	0.1434	1.17
DDC	50563851	rs11575342	A	0.136	0.122	0.124	0.4253	1.11	0.9285	0.99
DDC	50564124	rs3735274	G	0.240	0.255	0.228	0.5039	1.07	0.1472	1.16
DDC	50564358	rs3735273	A	0.232	0.206	0.262	0.1262	0.85	**0.0028**	0.73
DDC	50572526	rs11575322	A	0.082	0.107	0.080	0.8701	1.03	**0.0359**	1.39
DDC	50574882	rs998850	C	0.471	0.461	0.491	0.3734	0.92	0.1831	0.89
DDC	50578412	rs3779078	A	0.205	0.193	0.244	**0.0357**	0.80	**0.0056**	0.74
DDC	50579382	rs11575288	G	0.025	0.017	0.013	*0.0570*	1.99	0.3949	1.38
DDC	50579579	rs11575286	A	0.082	0.106	0.080	0.8701	1.03	**0.0424**	1.37
DDC	50580056	rs2044859	G	0.380	0.339	0.395	0.4794	0.94	**0.0097**	0.79

Table 17 Continued

DDC	50580400	rs7786398	G	0.461	0.444	0.475	0.5428	0.95	0.1732	0.88
DDC	50597258	rs3829897	A	0.374	0.371	0.383	0.6724	0.96	0.5831	0.95
STX1A	72752376	rs867500	C	0.347	0.348	0.346	0.9490	1.01	0.9331	1.01
STX1A	72759283	rs3793243	A	0.440	0.429	0.424	0.4422	1.07	0.7943	1.02
COPG2	129947065	rs10954272	G	0.228	0.226	0.215	0.4955	1.08	0.5629	1.07
COPG2	129955068	rs6967801	A	0.233	0.232	0.223	0.5900	1.06	0.6302	1.05
COPG2	129965829	rs13241924	A	0.461	0.462	0.442	0.4162	1.08	0.3837	1.08
COPG2	129984917	rs10954274	A	0.052	0.047	0.059	0.5230	0.88	0.2351	0.79
COPG2	129992573	rs3857855	G	0.158	0.152	0.150	0.6007	1.07	0.8952	1.02
COPG2	129993518	rs11763462	G	0.152	0.147	0.147	0.7554	1.04	0.9760	1.00
SLC18A1	20046706	rs1497020	G	0.319	0.287	0.303	0.4496	1.08	0.4456	0.93
SLC18A1	20049580	rs1018079	G	0.302	0.324	0.295	0.7315	1.04	0.1536	1.15
SLC18A1	20049719	rs10102779	C	0.252	0.263	0.256	0.8630	0.98	0.7159	1.04
SLC18A1	20049834	rs17092104	C	0.103	0.075	0.111	0.5277	0.91	**0.0047**	0.64
SLC18A1	20049996	rs903997	C	0.231	0.233	0.218	0.5072	1.08	0.4157	1.09
SLC18A1	20050350	rs4921692	G	0.096	0.088	0.111	0.2563	0.85	*0.0833*	0.77
SLC18A1	20052292	rs2270650	A	0.374	0.385	0.362	0.5712	1.05	0.2751	1.11
SLC18A1	20052435	rs17092107	A	0.100	0.076	0.103	0.8165	0.97	**0.0395**	0.72
SLC18A1	20063898	rs1390942	A	0.169	0.173	0.168	0.9439	1.01	0.7653	1.04
SLC18A1	20073883	rs13258461	A	0.457	0.461	0.464	0.7547	0.97	0.8735	0.99
SLC18A1	20076383	rs3779672	G	0.194	0.179	0.196	0.8894	0.98	0.3233	0.89
SLC18A1	20076626	rs3779673	A	0.167	0.155	0.177	0.5470	0.93	0.1807	0.85
SLC18A1	20080513	rs2279709	A	0.450	0.439	0.457	0.7478	0.97	0.4134	0.93
SLC18A1	20080993	rs1390938	A	0.248	0.248	0.258	0.6000	0.95	0.5981	0.95
SLC18A1	20081107	rs2270637	C	0.184	0.181	0.187	0.8858	0.98	0.7337	0.96
SLC18A1	20082746	rs2270641	C	0.353	0.374	0.349	0.8663	1.02	0.2500	1.11
SLC18A1	20082870	rs2270642	A	0.349	0.372	0.343	0.7770	1.03	0.1851	1.13
SLC18A1	20084992	rs1390939	A	0.472	0.457	0.476	0.8493	0.98	0.3971	0.93
SLC18A1	20085581	rs988713	G	0.247	0.249	0.256	0.6407	0.95	0.7111	0.96
SLC18A1	20086029	rs7836907	T	0.134	0.137	0.139	0.7410	0.96	0.8611	0.98
SLC18A1	20087205	rs2173114	C	0.497	0.491	0.479	0.4222	1.08	0.5807	1.05
SLC18A1	20088916	rs7820517	A	0.189	0.172	0.204	0.3739	0.91	*0.0644*	0.81
NEF3	24823733	rs11782211	G	0.073	0.096	0.073	0.9773	1.00	*0.0750*	1.34
NEF3	24824747	rs10096842	A	0.081	0.064	0.062	0.1077	1.33	0.8391	1.04
NEF3	24825500	rs196868	G	0.122	0.110	0.104	0.1804	1.20	0.6558	1.07
NEF3	24825757	rs1457266	A	0.358	0.366	0.335	0.2945	1.10	0.1506	1.15
NEF3	24830588	rs196864	C	0.068	0.074	0.074	0.6000	0.91	0.9449	0.99
NEF3	24832328	rs12515	A	0.165	0.152	0.159	0.7283	1.04	0.6518	0.95
NEF3	24834861	rs13251967	G	0.370	0.353	0.327	**0.0448**	1.21	0.2252	1.12
NEF3	24836483	rs2975180	G	0.365	0.361	0.341	0.2500	1.11	0.3327	1.10
Hey1	80836175	rs2461056	A	0.174	0.182	0.177	0.8405	0.98	0.7936	1.03
Hey1	80836720	rs6473177	G	0.092	0.075	0.095	0.7906	0.96	*0.0946*	0.76
Hey1	80837832	rs6986945	C	0.198	0.205	0.188	0.5451	1.07	0.3404	1.11
Hey1	80839084	rs1046472	A	0.271	0.294	0.264	0.7154	1.04	0.1441	1.16
Hey1	80841420	rs960978	A	0.312	0.316	0.318	0.7925	0.98	0.9188	0.99
Hey1	80845876	rs2467779	A	0.165	0.172	0.166	0.9749	1.00	0.7000	1.05
Hey1	80846477	rs2920950	A	0.255	0.240	0.267	0.5517	0.94	0.1679	0.87
FREQ	131975772	rs3780708	A	0.327	0.299	0.330	0.8921	0.99	0.1363	0.87
FREQ	131976625	rs1017112	G	0.196	0.218	0.194	0.8895	1.02	0.1844	1.16
FREQ	131984366	rs7849345	A	0.495	0.470	0.491	0.8351	1.02	0.3671	0.92
FREQ	131987341	rs4424362	A	0.322	0.340	0.289	0.1164	1.17	**0.0142**	1.27

Table 17 Continued

FREQ	131988371	rs3824544	A	0.239	0.259	0.225	0.4441	1.08	*0.0742*	1.21
FREQ	131995274	rs10819611	G	0.326	0.353	0.301	0.2171	1.13	**0.0135**	1.27
FREQ	131996050	rs1009502	A	0.310	0.353	0.301	0.6439	1.05	**0.0135**	1.27
FREQ	132003939	rs11793619	C	0.125	0.127	0.116	0.5408	1.09	0.4611	1.11
FREQ	132008905	rs870811	A	0.427	0.470	0.432	0.8225	0.98	*0.0840*	1.17
FREQ	132012650	rs947514	A	0.069	0.054	0.058	0.2833	1.22	0.7589	0.94
FREQ	132013809	rs947513	G	0.475	0.473	0.448	0.2260	1.11	0.2668	1.11
FREQ	132014745	rs10819615	A	0.326	0.313	0.335	0.6730	0.96	0.2761	0.90
FREQ	132018298	rs4240447	A	0.302	0.328	0.274	0.1606	1.15	**0.0094**	1.29
FREQ	132024932	rs3829905	A	0.407	0.420	0.377	0.1659	1.13	*0.0535*	1.19
FREQ	132028530	rs2277200	C	0.330	0.360	0.315	0.4453	1.08	**0.0301**	1.23
FREQ	132032983	rs7873936	A	0.211	0.196	0.176	*0.0524*	1.25	0.2542	1.14
FREQ	132034981	rs7852859	G	0.140	0.142	0.117	0.1335	1.23	0.1096	1.24
FREQ	132035589	rs1054879	A	0.495	0.498	0.503	0.7261	0.97	0.9561	1.01
FREQ	132036468	rs6478954	A	0.311	0.316	0.298	0.5240	1.06	0.3698	1.09
FREQ	132037596	rs13710	G	0.312	0.278	0.299	0.4997	1.07	0.3110	0.90
FREQ	132038378	rs11552451	G	0.146	0.141	0.157	0.4940	0.92	0.2942	0.88
DBH	135486529	rs3025373	G	0.141	0.142	0.148	0.6858	0.95	0.7342	0.96
DBH	135487964	rs1076153	A	0.176	0.200	0.180	0.7954	0.97	0.2581	1.14
DBH	135488582	rs1076150	A	0.468	0.512	0.483	0.4957	0.94	0.2070	1.12
DBH	135490336	rs1611115	A	0.198	0.229	0.217	0.2945	0.89	0.5369	1.07
DBH	135491762	rs2797849	G	0.353	0.327	0.336	0.4338	1.08	0.6635	0.96
DBH	135492142	rs3025382	A	0.131	0.119	0.133	0.8891	0.98	0.3257	0.88
DBH	135493077	rs3025388	G	0.186	0.167	0.177	0.6227	1.06	0.5601	0.93
DBH	135493640	rs2007153	A	0.380	0.393	0.393	0.5419	0.95	0.9975	1.00
DBH	135494744	rs1611118	A	0.053	0.061	0.065	0.2528	0.81	0.7265	0.94
DBH	135494935	rs1108580	A	0.439	0.479	0.442	0.8937	0.99	0.1014	1.16
DBH	135495062	rs1108581	G	0.216	0.205	0.225	0.6335	0.95	0.2582	0.88
DBH	135498795	rs3025399	C	0.044	0.049	0.030	0.1160	1.46	**0.0364**	1.64
DBH	135498904	rs1611123	A	0.503	0.453	0.489	0.5240	1.06	0.1140	0.87
DBH	135500715	rs2797855	G	0.429	0.392	0.434	0.8203	0.98	*0.0567*	0.84
DBH	135501206	rs1541333	G	0.438	0.470	0.443	0.8274	0.98	0.2266	1.12
DBH	135501337	rs1541332	A	0.448	0.454	0.428	0.3806	1.09	0.2343	1.11
DBH	135502096	rs2519154	G	0.415	0.425	0.397	0.4514	1.08	0.2170	1.12
DBH	135502336	rs2797853	A	0.324	0.332	0.347	0.2786	0.90	0.4726	0.93
DBH	135504489	rs6479643	G	0.402	0.404	0.382	0.3466	1.09	0.2962	1.10
DBH	135505151	rs2283124	A	0.132	0.115	0.123	0.5348	1.09	0.5842	0.93
DBH	135507918	rs77905	A	0.470	0.491	0.502	0.1484	0.88	0.6326	0.96
DBH	135510103	rs2073833	G	0.436	0.421	0.413	0.3182	1.10	0.7288	1.03
DBH	135512008	rs1611131	G	0.285	0.287	0.281	0.8548	1.02	0.7707	1.03
DBH	135512749	rs2073837	A	0.295	0.305	0.294	0.9393	1.01	0.5759	1.06
DBH	135513490	rs129882	A	0.187	0.201	0.211	0.1765	0.86	0.5898	0.94
DBH	135514632	rs129883	G	0.307	0.311	0.306	0.9500	1.01	0.8229	1.02
DBH	135514739	rs129915	G	0.288	0.292	0.277	0.5690	1.06	0.4659	1.08
DBH	135514907	rs129884	A	0.179	0.192	0.189	0.5592	0.94	0.8608	1.02
DBH	135518542	rs129886	A	0.193	0.205	0.214	0.2280	0.88	0.6114	0.95
SLC18A2	118995757	rs363393	T	0.182	0.186	0.195	0.4670	0.92	0.6270	0.95
SLC18A2	118998861	rs363399	G	0.239	0.243	0.268	0.1313	0.86	0.1997	0.88
SLC18A2	118999379	rs363338	G	0.302	0.319	0.326	0.2262	0.89	0.7494	0.97
SLC18A2	119004013	rs2072362	G	0.090	0.098	0.118	**0.0390**	0.74	0.1671	0.82
SLC18A2	119004938	rs363343	C	0.171	0.173	0.201	*0.0769*	0.82	0.1136	0.83

Table 17 Continued

SLC18A2	119008669	rs2283138	G	0.102	0.108	0.121	0.1606	0.82	0.3791	0.88
SLC18A2	119009116	rs929493	G	0.158	0.169	0.190	*0.0578*	0.80	0.2320	0.87
SLC18A2	119009457	rs363251	G	0.367	0.398	0.394	0.2120	0.89	0.8703	1.02
SLC18A2	119009648	rs11197936	G	0.393	0.352	0.366	0.2139	1.12	0.5140	0.94
SLC18A2	119011397	rs10082463	C	0.089	0.093	0.091	0.8735	0.98	0.9053	1.02
SLC18A2	119012563	rs363224	C	0.445	0.420	0.432	0.5598	1.06	0.5824	0.95
SLC18A2	119015202	rs363226	G	0.324	0.301	0.315	0.6526	1.04	0.5191	0.94
SLC18A2	119027061	rs14240	A	0.492	0.449	0.486	0.8229	1.02	*0.0972*	0.86
SLC18A2	119027658	rs363282	G	0.143	0.140	0.146	0.8746	0.98	0.6956	0.95
SLC18A2	119028361	rs363236	G	0.143	0.140	0.145	0.9127	0.99	0.7309	0.96
SLC18A2	119028749	rs363238	A	0.110	0.114	0.103	0.5841	1.08	0.4278	1.12
SLC18A2	119029149	rs363285	C	0.273	0.248	0.268	0.8048	1.03	0.2895	0.90
SLC18A2	119033544	rs363294	A	0.072	0.059	0.072	0.9845	1.00	0.2386	0.81
DRD1IP	134990001	rs7475905	A	0.026	0.023	0.030	0.5044	0.84	0.2963	0.75
DRD1IP	134991562	rs2298122	C	0.189	0.179	0.219	0.1035	0.83	**0.0261**	0.78
DRD1IP	134996704	rs11101694	G	0.129	0.127	0.169	**0.0141**	0.73	**0.0087**	0.72
DRD4	626399	rs3758653	G	0.185	0.194	0.177	0.6412	1.05	0.3482	1.11
DRD4	633568	rs936465	C	0.470	0.500	0.456	0.5338	1.06	**0.0487**	1.19
TH	2137971	rs3842748	C	0.232	0.240	0.234	0.9260	0.99	0.7841	1.03
TH	2142911	rs2070762	G	0.509	0.487	0.472	0.1105	1.16	0.5048	1.06
TH	2147527	rs6356	A	0.354	0.391	0.354	0.9848	1.00	*0.0900*	1.17
TH	2150751	rs10743149	A	0.127	0.123	0.106	0.1419	1.23	0.2413	1.18
TH	2150966	rs10840491	A	0.156	0.118	0.134	0.1751	1.19	0.2636	0.86
TH	2151386	rs7119275	A	0.379	0.382	0.381	0.8998	0.99	0.9594	1.01
TH	2154012	rs4929966	C	0.270	0.265	0.277	0.7247	0.97	0.5302	0.94
GRK2	66789652	rs11605263	A	0.045	0.038	0.049	0.6197	0.90	0.2273	0.77
GRK2	66806868	rs2071007	G	0.082	0.077	0.072	0.4372	1.14	0.6647	1.08
DRD2	112783693	rs2234689	G	0.188	0.177	0.178	0.5767	1.07	0.9537	0.99
DRD2	112783974	rs1554929	G	0.474	0.459	0.472	0.9027	1.01	0.5663	0.95
DRD2	112786283	rs6279	G	0.289	0.295	0.298	0.6816	0.96	0.9111	0.99
DRD2	112787485	rs1124492	A	0.101	0.116	0.120	0.1705	0.82	0.7911	0.96
DRD2	112788022	rs1079594	C	0.183	0.160	0.173	0.5487	1.07	0.4246	0.91
DRD2	112792088	rs2440390	A	0.131	0.123	0.121	0.4916	1.10	0.8494	1.03
DRD2	112797422	rs2587548	G	0.419	0.401	0.416	0.8826	1.01	0.4792	0.94
DRD2	112812339	rs4586205	C	0.242	0.250	0.247	0.7768	0.97	0.9118	1.01
DRD2	112814829	rs4620755	A	0.099	0.117	0.122	*0.1005*	0.79	0.7323	0.95
DRD2	112815079	rs11214606	A	0.058	0.038	0.048	0.3414	1.21	0.2719	0.78
DRD2	112815891	rs7125415	A	0.081	0.089	0.102	*0.0986*	0.78	0.3308	0.86
DRD2	112822277	rs17529477	A	0.295	0.326	0.337	**0.0389**	0.83	0.6191	0.95
DRD2	112822955	rs17601612	C	0.347	0.377	0.387	*0.0607*	0.84	0.6417	0.96
DRD2	112823618	rs4936270	A	0.106	0.099	0.102	0.7343	1.05	0.8672	0.98
DRD2	112824662	rs4274224	G	0.449	0.474	0.486	*0.0908*	0.86	0.5829	0.95
DRD2	112829684	rs4581480	G	0.103	0.097	0.102	0.8971	1.02	0.7018	0.94
DRD2	112834984	rs7131056	A	0.471	0.428	0.428	**0.0476**	1.19	0.9775	1.00
DRD2	112836742	rs4648317	A	0.137	0.140	0.128	0.5696	1.08	0.4431	1.11
DRD2	112839419	rs4630328	A	0.344	0.372	0.382	*0.0708*	0.85	0.6561	0.96
DRD2	112846601	rs4938019	G	0.138	0.141	0.129	0.5731	1.08	0.4481	1.11
DRD2	112852165	rs12364283	G	0.080	0.080	0.067	0.2855	1.20	0.2593	1.21
DRIP78	54502337	rs12308277	A	0.023	0.029	0.028	0.4436	0.80	0.9594	1.01
syngr3	1979309	rs2283476	A	0.147	0.148	0.156	0.5688	0.93	0.6186	0.94
syngr3	1983819	rs3183175	A	0.063	0.071	0.070	0.4954	0.88	0.9642	1.01

Table 17 Continued

HIC5	31391123	rs11646911	A	0.466	0.436	0.467	0.9430	0.99	0.1577	0.88
HIC5	31396534	rs13143	A	0.259	0.264	0.248	0.5865	1.06	0.4298	1.08
dopamineR PP32	35035375	rs879606	A	0.193	0.166	0.169	0.1676	1.18	0.8550	0.98
PPP1R9B	45563273	rs847682	A	0.475	0.479	0.490	0.5250	0.94	0.1591	0.88
PPP1R9B	45571943	rs1569116	A	0.142	0.144	0.136	0.6905	1.05	0.6030	1.07
PPP1R9B	45575583	rs4794103	A	0.180	0.177	0.164	0.3314	1.13	0.4281	1.10
PPP1R9B	45576919	rs12453363	A	0.157	0.179	0.139	0.2768	1.15	**0.0156**	1.35
PPP1R9B	45579074	rs847680	G	0.147	0.166	0.177	*0.0701*	0.80	0.4977	0.92
GRB2	70825380	rs17490675	G	0.049	0.061	0.067	*0.0935*	0.72	0.5902	0.91
GRB2	70826963	rs7219	G	0.239	0.253	0.274	*0.0817*	0.83	0.3045	0.90
GRB2	70839969	rs12600908	A	0.117	0.126	0.135	0.2113	0.84	0.5495	0.92
GRB2	70853307	rs4789172	G	0.454	0.486	0.491	0.1145	0.86	0.8265	0.98
GRB2	70855674	rs12950752	A	0.230	0.233	0.255	0.1944	0.87	0.2643	0.89
GRB2	70864463	rs4789176	A	0.159	0.165	0.187	0.1123	0.83	0.2134	0.86
GRB2	70867364	rs4350602	G	0.264	0.275	0.287	0.2446	0.89	0.5409	0.94
GRB2	70905054	rs2053158	A	0.169	0.184	0.206	**0.0387**	0.79	0.2162	0.87
SNAP25	10142678	rs8119844	A	0.293	0.274	0.304	0.5921	0.95	0.1444	0.87
SNAP25	10143433	rs6104567	C	0.263	0.245	0.244	0.3283	1.10	0.9790	1.00
SNAP25	10145086	rs1889189	A	0.323	0.355	0.326	0.8738	0.98	0.1764	1.14
SNAP25	10156748	rs3787303	G	0.174	0.188	0.161	0.4647	1.09	0.1141	1.21
SNAP25	10164902	rs363026	A	0.079	0.065	0.077	0.8665	1.03	0.3090	0.84
SNAP25	10165336	rs363011	G	0.089	0.118	0.083	0.6134	1.08	**0.0104**	1.47
SNAP25	10167799	rs363012	G	0.345	0.351	0.349	0.8660	0.98	0.9327	1.01
SNAP25	10168496	rs363039	A	0.358	0.317	0.343	0.4698	1.07	0.2280	0.89
SNAP25	10169467	rs363040	A	0.240	0.240	0.256	0.4091	0.92	0.4275	0.92
SNAP25	10174146	rs363043	A	0.288	0.288	0.325	*0.0735*	0.84	*0.0714*	0.84
SNAP25	10179174	rs363016	G	0.449	0.426	0.414	0.1067	1.15	0.5803	1.05
SNAP25	10183426	rs363052	A	0.207	0.215	0.214	0.6984	0.96	0.9450	1.01
SNAP25	10183926	rs3025866	A	0.029	0.023	0.024	0.4656	1.23	0.8637	0.95
SNAP25	10189811	rs363021	A	0.435	0.453	0.433	0.9422	1.01	0.3628	1.09
SNAP25	10191251	rs362563	G	0.049	0.064	0.041	0.3513	1.22	**0.0209**	1.61
SNAP25	10192626	rs362564	G	0.391	0.403	0.398	0.7346	0.97	0.8495	1.02
SNAP25	10193139	rs362547	A	0.422	0.425	0.447	0.2507	0.90	0.3236	0.91
SNAP25	10194091	rs362567	A	0.144	0.115	0.127	0.2493	1.16	0.4060	0.89
SNAP25	10194864	rs362570	A	0.196	0.180	0.166	*0.0740*	1.23	0.4046	1.10
SNAP25	10202475	rs362584	A	0.277	0.268	0.286	0.6478	0.96	0.3561	0.91
SNAP25	10217040	rs3025873	G	0.205	0.231	0.207	0.9306	0.99	0.1798	1.16
SNAP25	10217890	rs362549	G	0.476	0.495	0.491	0.5189	0.94	0.8347	1.02
SNAP25	10218925	rs362588	C	0.138	0.155	0.153	0.3174	0.88	0.8951	1.02
SNAP25	10224716	rs362993	A	0.089	0.101	0.081	0.4935	1.12	0.1101	1.28
SNAP25	10225621	rs362998	A	0.066	0.075	0.055	0.2659	1.23	*0.0691*	1.40
SNAP25	10228505	rs6108463	G	0.174	0.165	0.153	0.2033	1.16	0.4511	1.10
SNAP25	10229370	rs362988	A	0.480	0.436	0.451	0.1744	1.13	0.5061	0.94
SNAP25	10231950	rs6108464	G	0.409	0.378	0.380	0.1714	1.13	0.9330	0.99
SNAP25	10232418	rs3787283	G	0.360	0.328	0.348	0.5824	1.05	0.3426	0.91
SNAP25	10235742	rs8636	A	0.323	0.353	0.348	0.2356	0.89	0.8235	1.02
SNAP25	10238703	rs6074121	G	0.374	0.339	0.332	*0.0531*	1.20	0.7369	1.03
SNAP25	10239487	rs4813927	T	0.271	0.288	0.296	0.2313	0.89	0.7002	0.96
SNAP25	10239812	rs362599	C	0.340	0.352	0.352	0.5560	0.95	0.9856	1.00
SNAP25	10240769	rs6032846	G	0.398	0.413	0.414	0.4469	0.93	0.9622	1.00

Table 17 Continued

CLIC6	34982538	rs2236610	G	0.186	0.181	0.202	0.3520	0.90	0.2193	0.87
CLIC6	34991708	rs2834590	A	0.167	0.179	0.169	0.9147	0.99	0.5552	1.07
CLIC6	35002160	rs6517254	A	0.379	0.393	0.402	0.3184	0.91	0.6966	0.97
CLIC6	35002268	rs2070368	G	0.405	0.392	0.397	0.7235	1.03	0.8084	0.98
CLIC6	35007972	rs2834600	A	0.140	0.153	0.144	0.8385	0.97	0.5567	1.08
CLIC6	35010924	rs2834601	A	0.060	0.060	0.071	0.2999	0.83	0.3185	0.84
COMT	18307146	rs5748489	A	0.410	0.412	0.413	0.9170	0.99	0.9788	1.00
COMT	18308022	rs1800706	A	0.302	0.298	0.286	0.4392	1.08	0.5374	1.06
COMT	18310002	rs9306231	G	0.232	0.241	0.252	0.3167	0.90	0.5980	0.95
COMT	18310121	rs737865	G	0.304	0.301	0.284	0.3201	1.10	0.3897	1.09
COMT	18311407	rs933271	G	0.285	0.295	0.302	0.3949	0.92	0.7519	0.97
COMT	18313048	rs8185002	C	0.306	0.304	0.285	0.3256	1.10	0.3644	1.09
COMT	18313687	rs9332325	A	0.284	0.293	0.300	0.4262	0.93	0.7229	0.97
COMT	18314051	rs174675	A	0.284	0.293	0.300	0.4421	0.93	0.7229	0.97
COMT	18322484	rs9332347	A	0.121	0.130	0.136	0.2961	0.87	0.6810	0.95
COMT	18322997	rs5746849	A	0.468	0.465	0.464	0.8756	1.01	0.9851	1.00
COMT	18328337	rs4646312	G	0.407	0.396	0.415	0.7212	0.97	0.3845	0.92
COMT	18329644	rs3810595	G	0.411	0.400	0.423	0.5735	0.95	0.2822	0.91
COMT	18329952	rs6269	G	0.410	0.400	0.423	0.5451	0.95	0.2822	0.91
COMT	18330235	rs4633	G	0.485	0.492	0.496	0.6288	0.96	0.8781	0.99
COMT	18330428	rs2239393	G	0.410	0.400	0.423	0.5503	0.95	0.2857	0.91
COMT	18330763	rs740601	C	0.410	0.399	0.424	0.5189	0.94	0.2466	0.90
COMT	18331207	rs4818	C	0.406	0.392	0.416	0.6608	0.96	0.2747	0.91
COMT	18331271	rs4680	G	0.483	0.490	0.497	0.5373	0.95	0.7751	0.97
COMT	18331897	rs4646315	C	0.163	0.165	0.172	0.5967	0.94	0.6818	0.95
COMT	18332132	rs4646316	A	0.245	0.228	0.251	0.7680	0.97	0.2282	0.88
COMT	18332561	rs165774	A	0.332	0.315	0.308	0.2421	1.12	0.7358	1.03
COMT	18333176	rs174696	G	0.203	0.216	0.202	0.9679	1.01	0.4502	1.09
COMT	18333832	rs174697	A	0.054	0.055	0.049	0.6209	1.10	0.5358	1.13
COMT	18334458	rs174699	G	0.054	0.056	0.046	0.4024	1.18	0.2986	1.24
COMT	18335692	rs9332377	A	0.160	0.175	0.179	0.2738	0.88	0.8322	0.98
COMT	18336553	rs9332381	G	0.046	0.046	0.040	0.5238	1.15	0.4991	1.16
COMT	18336781	rs165599	G	0.306	0.340	0.304	0.9501	1.01	*0.0847*	1.18
COMT	18337023	rs165728	G	0.053	0.055	0.045	0.3924	1.19	0.2899	1.24
COMT	18337631	rs9265	C	0.306	0.339	0.305	0.9658	1.00	*0.0969*	1.17
COMT	18338669	rs165849	G	0.307	0.339	0.304	0.9046	1.01	*0.0907*	1.18
COMT	18339473	rs165815	G	0.138	0.156	0.125	0.3841	1.12	**0.0441**	1.30
ADRBK2	24261580	rs576895	G	0.259	0.252	0.275	0.4288	0.92	0.2591	0.89
ADRBK2	24264721	rs558934	G	0.280	0.290	0.296	0.4373	0.93	0.7712	0.97
ADRBK2	24280750	rs5761116	A	0.166	0.159	0.170	0.8031	0.97	0.4895	0.92
CACNG2	35288854	rs4820239	A	0.247	0.233	0.259	0.5309	0.94	0.1833	0.87
CACNG2	35306921	rs2267341	G	0.315	0.326	0.344	0.1721	0.88	0.3991	0.92
CACNG2	35316038	rs2283981	G	0.325	0.328	0.335	0.6020	0.95	0.7214	0.97
CACNG2	35322433	rs3788521	G	0.100	0.100	0.102	0.8741	0.98	0.9235	0.99
CACNG2	35338874	rs738977	A	0.226	0.227	0.246	0.2791	0.89	0.2987	0.90
CACNG2	35449746	rs738518	G	0.298	0.290	0.262	*0.0740*	1.20	0.1678	1.15
PICK1	36785415	rs713729	T	0.278	0.248	0.266	0.5575	1.06	0.3404	0.91
PICK1	36786544	rs3952	G	0.317	0.330	0.331	0.4923	0.94	0.9467	0.99
PICK1	36793598	rs2076369	A	0.373	0.394	0.375	0.9126	0.99	0.3927	1.08
PICK1	36803652	rs2012859	A	0.309	0.279	0.293	0.4427	1.08	0.4739	0.93
PICK1	36804642	rs2076371	A	0.256	0.272	0.263	0.7242	0.96	0.6714	1.04

Table 17 Continued

PICK1	36806222	rs8135665	A	0.193	0.217	0.219	0.1444	0.85	0.9112	0.99
MAOA	43411492	rs6520894	C	0.296	0.300	0.297	0.6536	0.99	0.9228	1.01
MAOA	43432254	rs5906957	A	0.255	0.255	0.243	0.8485	1.07	0.5921	1.07
MAOA	43436265	rs5906974	G	0.297	0.302	0.297	0.6861	1.00	0.8220	1.03
MAOA	43436344	rs3027392	A	0.045	0.029	0.030	0.2154	1.50	0.9044	0.96
MAOA	43438146	rs909525	G	0.328	0.326	0.324	0.8932	1.02	0.9211	1.01
MAOA	43477666	rs3027399	C	0.058	0.072	0.055	0.7881	1.05	0.1769	1.33
MAOA	43488335	rs1137070	A	0.301	0.302	0.308	0.5271	0.97	0.7863	0.97
MAOA	43489785	rs3027407	A	0.301	0.299	0.307	0.5718	0.97	0.7359	0.96
MAOB	43512943	rs1799836	G	0.455	0.445	0.421	*0.0623*	1.15	0.3602	1.10
MAOB	43536139	rs3027450	G	0.215	0.242	0.203	0.2903	1.07	*0.0707*	1.25
MAOB	43536455	rs2311013	A	0.043	0.052	0.044	0.4611	0.98	0.4519	1.20
MAOB	43559979	rs736944	A	0.165	0.151	0.178	0.9332	0.91	0.1593	0.82
MAOB	43562986	rs2283729	A	0.274	0.286	0.231	**0.0255**	1.26	**0.0136**	1.34
MAOB	43573908	rs6651806	C	0.284	0.304	0.243	*0.0570*	1.23	**0.0079**	1.36
MAOB	43588734	rs4824562	G	0.156	0.140	0.166	0.7147	0.93	0.1634	0.82
MAOB	43611338	rs5905512	G	0.505	0.495	0.475	**0.0189**	1.13	0.4448	1.08
FLNA	153231493	rs2070819	G	0.090	0.105	0.119	0.5685	0.73	0.3762	0.87
FLNA	153248612	rs2070816	G	0.196	0.211	0.199	0.4019	0.98	0.5663	1.08

Table 18 Supplementary Table 6.3: Hotellings T2 for all Genes

		Schizophrenia					Bipolar Disorder				
Gene	SNPs	T2	DF1	DF2	P_Hotel	EMP-P	T2	DF1	DF2	P_Hotel	EMP-P
ADRBK2	3	0.39	3	1000	0.757	0.756	0.48	3	996	0.694	0.700
CACNG2	6	1.33	6	997	0.242	0.247	1.07	6	993	0.379	0.374
CLIC6	6	0.35	5	998	0.879	0.881	0.55	5	994	0.736	0.734
COMT	31	0.68	15	988	0.804	0.804	0.94	14	985	0.511	0.518
COPG2	6	0.47	5	998	0.801	0.801	0.72	5	994	0.610	0.615
DARPP32	1	1.90	1	1002	0.168	0.179	0.03	1	998	0.855	0.849
DBH	29	0.88	20	983	0.616	0.617	0.65	20	979	0.877	0.878
DDC	38	0.99	15	988	0.465	0.391	0.77	15	984	0.712	0.708
DRD1	5	1.02	4	999	0.395	0.400	0.13	5	994	0.985	0.988
DRD1IP	3	2.20	3	1000	0.086	0.090	2.40	3	996	0.067	0.063
DRD2	21	1.01	12	991	0.438	0.433	0.63	12	987	0.819	0.813
DRD3	20	2.35	9	994	0.013	0.007	2.09	10	989	0.023	0.013
DRD4	2	0.29	2	1001	0.750	0.745	2.36	2	997	0.095	0.095
DRD5	1	0.28	1	1002	0.596	0.597	0.32	1	998	0.572	0.577
DRIP78	1	0.59	1	1002	0.444	0.496	0.00	1	998	0.962	1.000
EPB41	15	0.60	10	993	0.811	0.818	1.55	10	989	0.115	0.109
FLNA	2	2.93	2	1001	0.054	0.060	1.63	2	997	0.197	0.208
FREQ	21	0.74	18	985	0.774	0.776	1.08	18	981	0.373	0.376
GNB2L1	4	1.71	4	999	0.145	0.150	0.99	4	995	0.412	0.419
GRB2	8	0.92	7	996	0.489	0.373	0.58	5	994	0.715	0.715
GRK2	2	0.40	2	1001	0.668	0.671	0.80	2	997	0.451	0.447
Hey1	7	0.16	7	996	0.993	0.992	1.52	7	992	0.158	0.156
HIC5	2	0.19	2	1001	0.828	0.828	1.03	2	997	0.356	0.360
MAOA	8	1.17	5	998	0.323	0.336	0.93	5	994	0.463	0.481
MAOB	8	1.28	6	997	0.266	0.268	1.24	6	993	0.283	0.296
NCK1	6	0.37	4	999	0.832	0.826	0.11	4	995	0.977	0.977
NEF3	8	1.14	8	995	0.337	0.342	1.21	8	991	0.288	0.286
NR4A2	2	0.39	2	1001	0.680	0.682	0.43	2	997	0.649	0.643
PICK1	6	0.61	5	998	0.694	0.697	0.26	5	994	0.937	0.931
PPP1R9B	5	1.04	5	998	0.394	0.396	1.89	5	994	0.093	0.095
PPP2CA	1	0.40	1	1002	0.527	0.566	0.00	1	998	0.967	1.000
SLC18A1	22	0.72	14	989	0.754	0.761	1.46	14	985	0.119	0.113
SLC18A2	18	1.03	16	987	0.425	0.420	1.06	16	983	0.388	0.393
SLC6A3	44	1.00	24	979	0.470	0.479	0.73	23	976	0.820	0.818
SNAP25	34	1.10	25	978	0.333	0.334	1.06	25	974	0.381	0.375
SNCA	12	0.62	8	995	0.759	0.753	0.77	7	992	0.615	0.614
Sp4	3	2.05	3	1000	0.106	0.105	0.09	3	996	0.965	0.963
STX1A	2	0.57	2	1001	0.564	0.572	0.05	2	997	0.951	0.956
syngr3	2	0.43	2	1001	0.652	0.650	0.13	2	997	0.881	0.885
TH	7	1.29	7	996	0.254	0.247	1.28	7	992	0.258	0.252

Test statistics for all gene-based tests provided. P_Hotel = asymptotic p-value, EMP_P = empirical p-value from permutation.

Table 19 Supplementary Table 6.4: Most significant shared interactions

Gene Pair	SNP pair	SZ/SZA Ineraction p-value	SZ/SZA OR	BP1 Interaction p-value	BP1 OR
SLC6A3*COMT	rs1042098*rs9332347	0.004102	1.86	0.005004	1.83
SLC6A3*COMT	rs37022*rs5746849	0.008311	0.64	0.004138	0.61
SLC6A3*COMT	rs37022*rs3810595	0.008486	1.60	0.000169	1.95
SLC6A3*DDC	rs27072*rs1451372	0.001076	1.85	0.001355	1.80
SLC6A3*DDC	rs27072*rs730092	0.002693	1.75	0.002664	1.71
SLC6A3*DDC	rs11133770*rs1451372	0.003083	0.60	0.008423	0.64
SNCA*SLC6A3	rs2737020*rs40184	0.000918	1.68	0.000096	1.81
SNCA*SLC6A3	rs2737020*rs2963238	0.006455	1.54	0.009120	1.47
SNCA*SLC6A3	rs2737020*rs2937639	0.006456	1.55	0.005345	1.51
DDC*DRD2	rs4602840*rs6279	0.000067	0.33	0.002460	0.48
DDC*DRD2	rs11575548*rs6279	0.000116	0.34	0.001245	0.45
DDC*DRD2	rs4490786*rs6279	0.000774	0.53	0.001788	0.57
DDC*DRD2	rs11575500*rs6279	0.000826	0.35	0.008439	0.48
DDC*DRD2	rs11575500*rs1124492	0.001188	0.23	0.005118	0.36
DDC*DRD2	rs11575553*rs6279	0.001324	0.39	0.002275	0.43
DDC*DRD2	rs11575500*rs4620755	0.001455	0.23	0.005583	0.37
DDC*DRD2	rs11575322*rs6279	0.002127	0.43	0.002607	0.47
DDC*DRD2	rs11575286*rs6279	0.002127	0.43	0.003174	0.48
DDC*DRD2	rs4602840*rs4620755	0.002152	0.31	0.000542	0.31
DDC*DRD2	rs11575548*rs4586205	0.002337	0.42	0.008537	0.52
DDC*DRD2	rs11575548*rs4620755	0.003006	0.32	0.000272	0.28
DDC*DRD2	rs4602840*rs1124492	0.003031	0.33	0.000835	0.32
DDC*DRD2	rs11575548*rs1124492	0.004239	0.34	0.000433	0.29
DDC*DRD2	rs4602840*rs7125415	0.004374	0.30	0.000870	0.26
DDC*DRD2	rs11575500*rs7125415	0.005641	0.24	0.007194	0.30
DDC*DRD2	rs11575548*rs7125415	0.006342	0.31	0.000390	0.21
DDC*DRD2	rs3807558*rs4274224	0.007916	1.50	0.000106	1.88
DDC*DRD2	rs745043*rs4274224	0.008410	1.50	0.000082	1.90
DDC*DRD2	rs2044859*rs4274224	0.008429	1.41	0.009260	1.40
DRD3*SLC18A1	rs12636133*rs1390939	0.001761	0.67	0.009201	0.72
DRD3*SLC18A1	rs2046496*rs1390939	0.009455	1.40	0.005784	1.41
DRD3*SNCA	rs324035*rs356165	0.007760	1.59	0.005575	1.60
COPG2*DBH	rs3857855*rs1108580	0.001617	1.73	0.001275	1.81
COPG2*DBH	rs3857855*rs1541333	0.002250	1.70	0.004060	1.69
COPG2*DBH	rs3857855*rs2519154	0.002430	1.69	0.009847	1.62
COPG2*DBH	rs10954272*rs1076150	0.006521	1.53	0.004382	1.59
COPG2*DBH	rs10954272*rs1108580	0.007224	1.50	0.004646	1.55
COPG2*DBH	rs3857855*rs1541332	0.007514	1.59	0.007709	1.61
COPG2*DBH	rs3857855*rs1076150	0.007574	1.61	0.006381	1.70
COPG2*DBH	rs10954272*rs1541333	0.007772	1.49	0.009245	1.50
DBH*COMT	rs129884*rs4633	0.004273	0.62	0.008398	0.64
DBH*COMT	rs129884*rs4680	0.004626	0.62	0.007568	0.64
DBH*GRK2	rs1108580*rs2071007	0.004761	0.49	0.001629	0.44
DBH*SNAP25	rs129884*rs362549	0.000790	1.79	0.003504	1.64

Table 19 Continued

DDC*DBH	rs1451372*rs77905	0.000458	1.61	0.001339	1.54
DDC*DBH	rs1451372*rs129883	0.000769	1.64	0.005606	1.49
DDC*DBH	rs3807558*rs77905	0.001997	1.63	0.000812	1.72
DDC*DBH	rs3779078*rs77905	0.002108	1.63	0.003823	1.61
DDC*DBH	rs745043*rs77905	0.002535	1.61	0.001556	1.66
DDC*DBH	rs3807558*rs6479643	0.004665	0.63	0.002786	0.62
DDC*DBH	rs4470989*rs77905	0.005641	1.48	0.006906	1.47
DDC*DBH	rs745043*rs6479643	0.006343	0.64	0.005084	0.64
DDC*DBH	rs3735273*rs77905	0.007694	1.50	0.007153	1.52
DDC*DBH	rs3779078*rs6479643	0.008044	0.65	0.008674	0.65
DRD2*PPP1R9B	rs2234689*rs4794103	0.003995	0.54	0.001841	0.50
DRD2*SNAP25	rs4274224*rs3025873	0.000224	0.56	0.001435	0.61
DRD2*SNAP25	rs17601612*rs3025873	0.000824	0.56	0.006590	0.64
DRD2*SNAP25	rs4630328*rs362588	0.001034	0.51	0.006193	0.58
DRD2*SNAP25	rs4274224*rs362584	0.001533	1.58	0.002642	1.55
DRD2*SNAP25	rs4630328*rs3025873	0.001698	0.59	0.005333	0.63
DRD2*SNAP25	rs17529477*rs3025873	0.006320	0.62	0.001273	0.58
EPB41*COMT	rs203278*rs174696	0.001033	0.58	0.000444	0.56
EPB41*COMT	rs150089*rs174696	0.002086	0.59	0.001959	0.60
EPB41*COMT	rs575675*rs4646312	0.007096	1.86	0.003522	2.02
EPB41*COMT	rs575675*rs4818	0.008664	1.83	0.007585	1.89
EPB41*COPG2	rs150093*rs10954272	0.000775	0.46	0.009857	0.56
EPB41*COPG2	rs126013*rs10954272	0.004940	0.65	0.000676	0.60
EPB41*COPG2	rs10915216*rs10954272	0.006974	1.51	0.009130	0.67
EPB41*DBH	rs2762682*rs1611118	0.003655	0.36	0.007147	0.41
FREQ*TH	rs3829905*rs3842748	0.006040	0.65	0.000185	0.56
GNB2L1*syngr3	rs2261114*rs3183175	0.000727	2.47	0.007557	1.96
GRK2*COMT	rs2071007*rs174696	0.002992	0.43	0.001723	0.41
HIC5*SNAP25	rs11646911*rs362998	0.005128	2.34	0.000700	2.76
HIC5*SNAP25	rs11646911*rs362563	0.005143	2.71	0.002875	2.75
NCK1*SLC18A2	rs9845460*rs363226	0.006039	0.62	0.004956	0.60
NEF3*SLC18A2	rs196868*rs363238	0.001658	3.82	0.006270	3.13
NEF3*SLC18A2	rs196868*rs363224	0.007843	1.78	0.003565	1.89
NR4A2*SNAP25	rs12803*rs8636	0.002733	0.67	0.000473	0.61
SLC18A1*ADRBK2	rs2270641*rs558934	0.007900	0.67	0.004438	0.65
SLC18A1*ADRBK2	rs2270642*rs558934	0.008610	0.67	0.002566	0.63
SLC18A1*DBH	rs3779672*rs3025373	0.000273	2.30	0.005648	1.94
SLC18A1*DRD2	rs2270642*rs12364283	0.004614	2.21	0.007881	2.05
SLC18A1*FREQ	rs3779672*rs4424362	0.000105	2.05	0.002116	1.83
SLC18A1*FREQ	rs17092104*rs2277200	0.005870	1.86	0.008970	1.89
SLC18A1*FREQ	rs3779672*rs3824544	0.006086	1.71	0.004677	1.81
SNCA*TH	rs3822095*rs6356	0.000052	1.84	0.000119	1.70
SNCA*TH	rs3822095*rs7119275	0.000215	0.58	0.001377	0.64
TH*PICK1	rs3842748*rs3952	0.000271	0.56	0.003976	0.64

SNP pairs provided where interaction p < 0.001 in both disorders. OR = odds ratio. No interaction tests significant after corrections for multiple comparisons

Table 20 Supplementary Table 6.5: Most significant diagnosis specific interactions

SCHIZOPHRENIA					
gene-pair	SNP pair	Main Effect SNP1	Main Effect SNP2	SZ/SZA Ineraction p-value	SZ/SZA OR
SLC18A2*COMT	rs11197936*rs5748489	0.249	0.975	0.000234	0.60
SLC6A3*DBH	rs37022*rs129915	0.518	0.610	0.000343	1.92
SLC6A3*DBH	rs37022*rs1611131	0.518	0.901	0.000505	1.92
SLC6A3*DBH	rs11564764*rs1611123	0.729	0.524	0.000593	2.54
SLC6A3*DBH	rs28363119*rs2797855	0.942	0.839	0.000598	2.62
SLC6A3*DBH	rs11564764*rs2797855	0.729	0.839	0.000750	2.59
SLC6A3*DBH	rs11564764*rs1108580	0.729	0.845	0.000786	0.41
SLC6A3*DBH	rs28363119*rs1611123	0.942	0.524	0.000854	2.44
SLC6A3*DBH	rs6869645*rs1611123	0.810	0.524	0.000902	2.43
SLC6A3*DBH	rs6876225*rs1611123	0.738	0.524	0.000918	2.43
SLC6A3*HIC5	rs2078247*rs13143	0.662	0.657	0.000133	0.52
SLC6A3*HIC5	rs2455391*rs13143	0.821	0.657	0.000252	0.54
SLC6A3*TH	rs6347*rs10743149	0.826	0.098	0.000537	2.29
DDC*DRD2	rs4602840*rs6279	0.628	0.705	0.000067	0.33
DDC*DRD2	rs11575548*rs6279	0.564	0.705	0.000116	0.34
DDC*DRD2	rs11575453*rs17529477	0.705	0.037	0.000279	0.36
DDC*DRD2	rs11575438*rs17529477	0.705	0.037	0.000279	0.36
DDC*DRD2	rs4490786*rs6279	0.893	0.705	0.000774	0.53
DDC*DRD2	rs11575500*rs6279	0.416	0.705	0.000826	0.35
DDC*COMT	rs2044859*rs9265	0.505	0.981	0.000108	0.57
DDC*COMT	rs2044859*rs165599	0.505	0.997	0.000109	0.57
DDC*COMT	rs2044859*rs165849	0.505	0.957	0.000177	0.58
DDC*COMT	rs3735273*rs9265	0.141	0.981	0.000184	0.52
DDC*COMT	rs3735273*rs165599	0.141	0.997	0.000223	0.53
DDC*COMT	rs3735273*rs165849	0.141	0.957	0.000354	0.54
DDC*COMT	rs7786398*rs9265	0.551	0.981	0.000893	0.62
DDC*COMT	rs7786398*rs165599	0.551	0.997	0.000990	0.62
DDC*DBH	rs11575322*rs2797853	0.911	0.286	0.000239	2.75
DDC*DBH	rs11575286*rs2797853	0.911	0.286	0.000239	2.75
DDC*DBH	rs1451372*rs77905	0.067	0.177	0.000458	1.61
DDC*DBH	rs1451372*rs129883	0.067	0.931	0.000769	1.64
COPG2*SLC18A2	rs3857855*rs363343	0.652	0.073	0.000631	0.44
DBH*SNAP25	rs129884*rs362549	0.618	0.496	0.000790	1.79
DDC*ADRBK2	rs4602840*rs558934	0.628	0.518	0.000346	2.61
DDC*ADRBK2	rs11575548*rs558934	0.564	0.518	0.000427	2.61
DRD1*DBH	rs5326*rs2007153	0.234	0.541	0.000638	0.51
DRD1*PICK1	rs267416*rs8135665	0.847	0.150	0.000112	0.53
DRD1*PICK1	rs4867798*rs2012859	0.095	0.455	0.000838	0.61
DRD1*TH	rs5326*rs2070762	0.234	0.110	0.000166	0.51
DRD1IP*PICK1	rs11101694*rs2076371	0.012	0.722	0.000250	0.45
DRD2*SNAP25	rs4274224*rs3025873	0.078	0.987	0.000224	0.56
DRD2*SNAP25	rs17601612*rs362588	0.055	0.376	0.000640	0.50
DRD2*SNAP25	rs17601612*rs3025873	0.055	0.987	0.000824	0.56

Table 20 Continued

DRD3*SLC18A1	rs12636133*rs2270642	0.023	0.770	0.000616	1.59
DRD3*SLC18A1	rs12636133*rs2270641	0.023	0.860	0.000639	1.58
DRD3*SLC18A1	rs963468*rs2270642	0.030	0.770	0.000783	1.57
DRD3*SLC18A1	rs963468*rs2270641	0.030	0.860	0.000878	1.56
DRD3*SLC18A1	rs10934254*rs2270642	0.035	0.770	0.000956	1.56
DRD3*SLC18A1	rs3732790*rs2270642	0.033	0.770	0.000998	1.55
DRD3*SLC18A1	rs10934254*rs2270641	0.035	0.860	0.000999	1.56
EPB41*CACNG2	rs575675*rs2267341	0.165	0.164	0.000805	0.43
EPB41*CACNG2	rs150089*rs2267341	0.272	0.164	0.000870	0.62
EPB41*COPG2	rs150093*rs3857855	0.847	0.652	0.000387	0.37
EPB41*COPG2	rs150093*rs10954272	0.847	0.521	0.000775	0.46
EPB41*COPG2	rs150093*rs10954274	0.847	0.497	0.000888	0.18
EPB41*DBH	rs10915216*rs129882	0.713	0.203	0.000604	1.74
EPB41*DBH	rs126013*rs129882	0.322	0.203	0.000817	0.56
FREQ*COMT	rs10819611*rs9332377	0.226	0.242	0.000564	1.96
FREQ*DBH	rs4424362*rs1611131	0.122	0.901	0.000174	0.56
FREQ*DBH	rs4424362*rs2073837	0.122	0.989	0.000302	0.58
FREQ*DBH	rs4424362*rs129915	0.122	0.610	0.000620	0.59
FREQ*PICK1	rs11552451*rs2012859	0.487	0.455	0.000090	0.44
FREQ*PICK1	rs11552451*rs713729	0.487	0.532	0.000613	0.49
FREQ*SNAP25	rs3824544*rs4813927	0.440	0.265	0.000605	1.79
FREQ*SNAP25	rs11552451*rs362549	0.487	0.496	0.000994	1.84
GNB2L1*syngr3	rs2261114*rs3183175	0.327	0.468	0.000727	2.47
GNB2L1*TH	rs2261114*rs3842748	0.327	0.854	0.000750	1.62
Hey1*DRD2	rs2461056*rs7131056	0.952	0.037	0.000930	1.82
SLC18A1*DBH	rs3779672*rs3025373	0.824	0.636	0.000273	2.30
SLC18A1*DBH	rs3779673*rs3025373	0.497	0.636	0.000393	2.35
SLC18A1*FREQ	rs17092104*rs4424362	0.490	0.122	0.000076	2.66
SLC18A1*FREQ	rs3779672*rs4424362	0.824	0.122	0.000105	2.05
SLC18A1*FREQ	rs17092104*rs10819611	0.490	0.226	0.000288	2.39
SLC18A1*FREQ	rs3779673*rs4424362	0.497	0.122	0.000335	2.03
SLC18A1*FREQ	rs3779672*rs10819611	0.824	0.226	0.000457	1.89
SLC18A1*FREQ	rs17092107*rs10819611	0.773	0.226	0.000486	2.35
SLC18A1*FREQ	rs17092107*rs4424362	0.773	0.122	0.000604	2.36
SLC18A1*FREQ	rs17092107*rs3829905	0.773	0.156	0.000978	2.15
SLC18A1*FREQ	rs3779673*rs10819611	0.497	0.226	0.000997	1.89
SLC18A1*PPP1R9B	rs903997*rs12453363	0.470	0.235	0.000976	0.48
SNAP25*CLIC6	rs363043*rs2834590	0.086	0.855	0.000274	2.02
SNAP25*CLIC6	rs3025873*rs2834590	0.987	0.855	0.000599	2.15
SNAP25*COMT	rs6104567*rs737865	0.330	0.352	0.000042	0.51
SNAP25*COMT	rs6104567*rs1800706	0.330	0.477	0.000114	0.53
SNAP25*COMT	rs6104567*rs8185002	0.330	0.358	0.000139	0.53
SNAP25*COMT	rs8119844*rs737865	0.646	0.352	0.000416	1.77
SNAP25*COMT	rs8119844*rs1800706	0.646	0.477	0.000501	1.75
SNAP25*COMT	rs363016*rs737865	0.111	0.352	0.000642	0.60
SNAP25*COMT	rs363016*rs1800706	0.111	0.477	0.000713	0.60
SNCA*DRD1IP	rs17016274*rs11101694	0.822	0.012	0.000529	3.62
SNCA*FREQ	rs356186*rs870811	0.527	0.842	0.000572	0.56

Table 20 Continued

SNCA*FREQ	rs356186*rs947513	0.527	0.222	0.000576	0.56
SNCA*SLC6A3	rs2737020*rs40184	0.424	0.911	0.000918	1.68
SNCA*TH	rs3822095*rs6356	0.255	0.974	0.000052	1.84
SNCA*TH	rs3822095*rs7119275	0.255	0.933	0.000215	0.58
TH*PICK1	rs3842748*rs3952	0.854	0.505	0.000271	0.56
TH*PICK1	rs10743149*rs2076369	0.098	0.879	0.000617	0.46

BIPOLAR DISORDER

gene-pair	SNP pair	Main Effect SNP1	Main Effect SNP2	BP1 Interaction p-value	BP1 OR
SLC6A3*COMT	rs37022*rs4818	0.827	0.268	0.000051	2.07
SLC6A3*COMT	rs37022*rs4646312	0.827	0.376	0.000065	2.05
SLC6A3*COMT	rs37022*rs3810595	0.827	0.274	0.000169	1.95
SLC6A3*COMT	rs37022*rs6269	0.827	0.274	0.000169	1.95
SLC6A3*COMT	rs37022*rs2239393	0.827	0.278	0.000173	1.95
SLC6A3*COMT	rs37022*rs740601	0.827	0.239	0.000264	1.91
SLC6A3*COMT	rs464061*rs4818	0.510	0.268	0.000929	1.76
SLC6A3*COMT	rs464061*rs4646312	0.510	0.376	0.000936	1.76
DDC*DRD2	rs745043*rs4274224	0.005	0.583	0.000082	1.90
DDC*DRD2	rs3807558*rs4274224	0.010	0.583	0.000106	1.88
DDC*DRD2	rs11575553*rs4620755	0.168	0.733	0.000189	0.22
DDC*DRD2	rs11575548*rs4620755	0.042	0.733	0.000272	0.28
DDC*DRD2	rs3735273*rs4274224	0.003	0.583	0.000324	1.76
DDC*DRD2	rs11575548*rs7125415	0.042	0.325	0.000390	0.21
DDC*DRD2	rs3779078*rs4274224	0.005	0.583	0.000417	1.78
DDC*DRD2	rs11575548*rs1124492	0.042	0.793	0.000433	0.29
DDC*DRD2	rs3779078*rs4630328	0.005	0.652	0.000497	1.78
DDC*DRD2	rs4602840*rs4620755	0.054	0.733	0.000542	0.31
DDC*DRD2	rs11575553*rs7125415	0.168	0.325	0.000546	0.19
DDC*DRD2	rs11575553*rs1124492	0.168	0.793	0.000584	0.25
DDC*DRD2	rs11575500*rs4936270	0.059	0.867	0.000692	0.24
DDC*DRD2	rs3807558*rs4630328	0.010	0.652	0.000701	1.74
DDC*DRD2	rs745043*rs4630328	0.005	0.652	0.000718	1.74
DDC*DRD2	rs3779078*rs17601612	0.005	0.640	0.000797	1.74
DDC*DRD2	rs4602840*rs1124492	0.054	0.793	0.000835	0.32
DDC*DRD2	rs3807558*rs17601612	0.010	0.640	0.000851	1.72
DDC*DRD2	rs745043*rs17601612	0.005	0.640	0.000856	1.72
DDC*DRD2	rs4602840*rs7125415	0.054	0.325	0.000870	0.26
DDC*DRD2	rs11575500*rs4581480	0.059	0.702	0.000945	0.25
DDC*DRD2	rs3807558*rs17529477	0.010	0.610	0.001000	1.75
COPG2*DBH	rs13241924*rs3025382	0.404	0.331	0.000803	0.53
DDC*CACNG2	rs3807563*rs2267341	0.358	0.402	0.000239	1.69
DDC*DBH	rs3807558*rs77905	0.010	0.628	0.000812	1.72
DDC*SLC18A2	rs1451371*rs363399	0.183	0.196	0.000070	0.52
DDC*SLC18A2	rs1451371*rs363338	0.183	0.747	0.000215	0.58
DDC*SLC18A2	rs3807562*rs363399	0.298	0.196	0.000825	0.58
DRD3*DDC	rs2046496*rs11575453	0.017	0.799	0.000999	2.16

Table 20 Continued

DRD3*DDC	rs2046496*rs11575438	0.017	0.799	0.000999	2.16
EPB41*COMT	rs203278*rs174696	0.209	0.460	0.000444	0.56
EPB41*COMT	rs575675*rs174696	0.024	0.460	0.000489	0.36
EPB41*COPG2	rs126013*rs10954272	0.177	0.574	0.000676	0.60
EPB41*COPG2	rs126013*rs13241924	0.177	0.404	0.000820	0.65
NR4A2*Hey1	rs834834*rs6473177	0.836	0.090	0.000465	0.41
NR4A2*SNAP25	rs12803*rs8636	0.429	0.821	0.000473	0.61
SLC6A3*NEF3	rs37022*rs196864	0.827	0.944	0.000569	3.54
SLC6A3*SLC18A1	rs12516758*rs7820517	1.000	0.066	0.000739	0.48
SNCA*Hey1	rs10002435*rs2461056	0.415	0.791	0.000643	0.34
SNCA*SLC6A3	rs2737020*rs40184	0.159	0.238	0.000096	1.81
SNCA*TH	rs3822095*rs6356	0.192	0.094	0.000119	1.70

SNP pairs provided where interaction p < 0.001 in either disorder. Main effect = result of Armitage Trends test for individual SNP. OR = odds ratio. No interaction tests significant after corrections for multiple comparisons

Table 21 Supplementary Table 6.7: Exploratory analyses for DRD3 and DDC

		Overall			Gender Specific Analyses					Diagnosis specific analyses			
gene	SNP	pval (SZ)	pval (BP1)	Strat pval (SZ)	Strat pval (BP1)	Male pval (SZ)	Male OR (SZ)	Female pval (SZ)	Female OR (SZ)	SZ only (pval)	SZA only (pval)	BP1 + psych	BP1 No psych
DRD3	rs2046496	**0.0345**	**0.0166**	**0.0423**	**0.0149**	0.3866	1.11	**0.0368**	1.33	*0.0773*	*0.0904*	**0.0143**	0.5154
DRD3	rs12636133	**0.0239**	**0.0092**	**0.0301**	**0.0083**	0.4595	0.91	**0.0147**	0.71	*0.0613*	*0.0705*	**0.0137**	0.1199
DRD3	rs10934254	**0.0367**	**0.0092**	**0.0446**	**0.0093**	0.5532	0.93	**0.0182**	0.72	0.1048	*0.0705*	**0.0137**	0.1528
DRD3	rs9868039	**0.0039**	**0.0017**	**0.0032**	**0.0014**	0.1102	1.22	**0.0082**	1.44	**0.0027**	0.1175	**0.0010**	0.4615
DRD3	rs9817063	**0.0123**	**0.0032**	**0.0086**	**0.0035**	0.2455	0.87	**0.0078**	0.69	**0.0202**	*0.0859*	**0.0042**	0.1728
DRD3	rs3732790	**0.0334**	**0.0117**	**0.0366**	**0.0096**	0.7359	0.96	**0.0054**	0.68	**0.0375**	0.1935	**0.0179**	0.1304
DRD3	rs2134655	**0.0080**	**0.0052**	**0.0065**	**0.0056**	0.3272	1.15	**0.0029**	1.59	**0.0075**	0.1100	**0.0036**	0.8739
DRD3	rs963468	**0.0302**	**0.0082**	**0.0336**	**0.0076**	0.6270	0.94	**0.0077**	0.69	**0.0388**	0.1645	**0.0146**	0.1434
DRD3	rs324035	0.4879	0.8727	0.5898	0.8838	0.5932	1.08	0.8349	1.04	0.7456	0.3745	0.8677	0.1733
DRD3	rs2630351	0.2630	0.1154	0.3788	0.1557	0.1747	1.41	0.8007	0.93	0.5585	0.1586	0.3912	**0.02643**
DRD3	rs167771	0.4593	0.6263	0.5956	0.6244	0.5000	1.11	0.9755	1.01	0.5922	0.4784	0.8919	0.1337
DRD3	rs324032	0.1492	*0.0818*	0.2351	0.1148	0.1059	1.50	0.9362	0.98	0.3380	0.1156	0.2815	**0.02643**
DRD3	rs167770	0.2323	0.2301	0.2159	0.2609	0.1336	0.82	0.8689	0.98	0.2698	0.4104	0.2482	0.7912
DRD3	rs226082	0.2537	0.2489	0.2444	0.2807	0.1200	0.81	0.9952	1.00	0.3374	0.3613	0.2482	0.6827
DRD3	rs324030	0.2537	0.2489	0.2444	0.2807	0.1200	0.81	0.9952	1.00	0.3374	0.3613	0.2482	0.6827
DRD3	rs7625282	*0.0575*	*0.0726*	*0.0527*	*0.0892*	**0.0372**	0.75	0.5789	0.92	**0.0477**	0.3092	*0.0533*	0.6532
DRD3	rs10934256	**0.0355**	0.1322	**0.0444**	0.1470	**0.0281**	0.72	0.5837	0.91	**0.0317**	0.2435	*0.0948*	0.6067
DRD3	rs7633291	**0.0463**	0.1467	*0.0578*	0.1621	**0.0331**	0.73	0.6525	0.93	**0.0470**	0.2435	*0.0948*	0.4985
DRD3	rs6280	0.5569	0.6806	0.4866	0.6872	0.2817	0.87	0.8619	1.03	0.5934	0.6767	0.5691	0.3388
DRD3	rs1800828	0.1651	0.5234	0.1601	0.5121	0.1154	0.80	0.7411	0.95	0.1163	0.5581	0.3316	0.271
DDC	rs11575564	*0.0602*	0.7525	*0.0951*	0.7327	0.1742	1.51	0.3267	1.41	0.2594	**0.0267**	0.7739	0.5501
DDC	rs4947510	0.2989	0.2375	0.3197	0.2542	0.2170	0.85	0.9204	0.99	0.4848	0.2925	0.4082	0.6053
DDC	rs11575553	0.9700	0.1576	0.7312	0.1504	0.3335	0.82	0.4630	1.23	0.9412	0.9810	**0.0441**	0.5413
DDC	rs11575548	0.5770	**0.0399**	0.8216	**0.0342**	0.2693	0.80	*0.0731*	1.58	0.6213	0.6710	**0.0031**	0.3877
DDC	rs11575542	0.3810	0.5460	0.2749	0.4867	0.6779	1.21	0.2753	1.56	0.3840	0.5847	0.5986	0.4619
DDC	rs4947535	**0.0339**	**0.0325**	*0.0532*	**0.0292**	0.3043	0.88	*0.0792*	0.77	0.1027	*0.0591*	**0.0078**	0.9468
DDC	rs11575535	0.2255	0.5405	0.1810	0.4892	0.3410	1.46	0.3468	1.42	0.1502	0.6628	0.5066	0.8114

Table 21 Continued

DDC	rs730092	0.1248	0.2787	0.1528	0.2625	0.1470	0.84	0.6040	0.93	0.2508	0.1564	0.3192	0.5645
DDC	rs11575500	0.4383	*0.0637*	0.5005	*0.0536*	0.7402	0.93	0.1475	1.50	0.4517	0.6084	**0.0080**	0.4719
DDC	rs745043	**0.0243**	**0.0037**	**0.0448**	**0.0037**	0.1278	0.80	0.1909	0.81	*0.0697*	*0.0594*	**0.0046**	0.5208
DDC	rs4490786	0.8721	*0.0592*	0.9104	*0.0685*	0.7111	0.95	0.7893	1.05	0.7379	0.8922	*0.0558*	0.9236
DDC	rs11575453	0.7460	0.7190	0.6849	0.8167	0.6729	1.09	0.2664	0.76	0.9644	0.4941	0.6079	0.4463
DDC	rs11575441	0.7219	0.6546	0.8978	0.7057	0.7267	1.19	0.7903	0.83	0.4520	0.7216	0.8074	0.9045
DDC	rs1451371	0.2400	0.1927	0.3190	0.1801	0.3243	1.13	0.6972	1.06	0.1379	0.7552	0.1681	0.6151
DDC	rs11575438	0.7460	0.7190	0.6849	0.8167	0.6729	1.09	0.2664	0.76	0.9644	0.4941	0.6079	0.4463
DDC	rs1451372	*0.0622*	0.3277	*0.0671*	0.3255	0.1080	0.82	0.3444	0.88	0.1694	*0.0783*	0.3684	0.5777
DDC	rs4470989	**0.0275**	**0.0284**	**0.0439**	**0.0260**	0.3300	0.88	*0.0526*	0.75	0.1063	**0.0376**	**0.0053**	0.9016
DDC	rs4602840	0.6406	*0.0513*	0.9077	**0.0443**	0.3319	0.83	0.1480	1.44	0.7726	0.6082	**0.0063**	0.4511
DDC	rs6957607	0.8475	0.7164	0.7338	0.8180	0.5756	1.13	0.2302	0.73	0.8517	0.5336	0.6275	0.3532
DDC	rs3807563	0.8091	0.3693	0.9546	0.3623	0.9336	1.01	0.9931	1.00	0.6131	0.8451	0.2023	0.7971
DDC	rs3807562	0.1768	0.3140	0.2154	0.2991	0.3486	1.12	0.4179	1.12	0.1074	0.6356	0.2616	0.8751
DDC	rs11575387	0.7925	0.8888	0.6855	0.9907	0.6949	1.09	0.2718	0.75	0.8810	0.4778	0.6125	0.6158
DDC	rs3807558	**0.0365**	**0.0079**	*0.0663*	**0.0080**	0.1506	0.81	0.2505	0.83	*0.0997*	*0.0717*	**0.0104**	0.561
DDC	rs11575375	0.5059	0.2259	0.5016	0.2779	0.8013	1.03	0.4659	1.11	0.9683	0.2297	0.2528	0.9477
DDC	rs4947584	0.4817	0.1799	0.4799	0.2252	0.7234	1.05	0.5055	1.10	0.9503	0.2128	0.2492	0.7485
DDC	rs6592961	0.6377	0.1511	0.8346	0.1737	0.7653	0.96	0.4987	1.13	0.5866	0.8496	0.1205	0.912
DDC	rs10274275	0.4946	0.1270	0.5007	0.1430	0.6051	1.08	0.6658	1.07	0.9719	0.2131	0.1362	0.5077
DDC	rs11575342	0.4253	0.9429	0.3396	0.9030	0.2370	1.25	0.8877	1.03	0.7990	*0.0735*	0.6266	0.4954
DDC	rs3735274	0.5039	0.1319	0.5177	0.1465	0.5864	1.08	0.7178	1.06	0.9316	0.2483	0.1327	0.5511
DDC	rs3735273	0.1262	**0.0024**	0.2140	**0.0024**	0.2016	0.83	0.6569	0.93	0.1876	0.2396	**0.0029**	0.4721
DDC	rs11575322	0.8701	**0.0306**	0.8564	**0.0303**	0.2763	0.80	0.2724	1.34	0.9086	0.8718	**0.0093**	0.8299
DDC	rs998850	0.3734	0.1608	0.5277	0.1707	0.4798	0.92	0.8770	0.98	0.2342	0.8738	0.1750	0.8719
DDC	rs3779078	**0.0357**	**0.0043**	*0.0662*	**0.0051**	0.1189	0.80	0.3102	0.85	*0.0864*	*0.0836*	**0.0057**	0.4946
DDC	rs11575288	*0.0570*	0.4301	0.1062	0.3806	**0.0231**	3.27	0.9957	1.00	*0.0730*	0.1501	0.7891	0.1738
DDC	rs11575286	0.8701	**0.0365**	0.8564	**0.0355**	0.2763	0.80	0.2724	1.34	0.9086	0.8718	**0.0119**	0.8299
DDC	rs2044859	0.4794	**0.0097**	0.7542	**0.0081**	0.7650	0.96	0.8917	0.98	0.2337	0.8287	**0.0051**	0.8865
DDC	rs7786398	0.5428	0.1598	0.6897	0.1691	0.3661	0.90	0.6766	1.06	0.2619	0.7674	0.1664	0.977
DDC	rs3829897	0.6724	0.5304	0.8005	0.5649	0.5342	0.93	0.7501	1.05	0.9209	0.5028	0.9031	0.7039

Table 22 Supplementary Table 6.7 Allele frequencies of 'alternate controls' for shared risk loci

Genomic			Association Results				Current Study Frequencies				Frequencies from other samples	
Gene	SNP	Position	BP1 Trends p-value	BP1 OR	SZ Trends p-value	SZ OR	N	SZ Case	BP1 Case	Control	HapMap CEU	ALT Controls
DDC	rs4947535	50499175	0.033	0.82	0.036	0.82	A	0.296	0.295	0.340	0.350	
DDC	rs745043	50511449	0.004	0.73	0.029	0.78	A	0.206	0.195	0.248	0.207	
DDC	rs4470989	50530192	0.028	0.81	0.029	0.81	A	0.297	0.297	0.343		0.350**
DDC	rs3807558	50538516	0.008	0.75	0.043	0.8	A	0.207	0.198	0.246	0.202	
DDC	rs3779078	50578412	0.004	0.74	0.042	0.8	A	0.205	0.193	0.244	0.200	0.200**
DRD1IP	rs11101694	134996704	0.008	0.72	0.012	0.73	G	0.129	0.127	0.169	0.150	
DRD3	rs2046496	115317621	0.017	1.24	0.031	1.21	G	0.494	0.502	0.448	0.475	0.480*
DRD3	rs12636133	115322414	0.009	0.79	0.023	0.81	C	0.424	0.416	0.475		0.436*
DRD3	rs10934254	115324324	0.009	0.79	0.035	0.83	G	0.428	0.417	0.475		0.438*
DRD3	rs9868039	115329232	0.002	1.34	0.003	1.30	A	0.451	0.458	0.387		0.450*
DRD3	rs9817063	115329798	0.003	0.77	0.011	0.79	G	0.456	0.447	0.514	0.475	0.453*
DRD3	rs3732790	115329973	0.012	0.79	0.033	0.82	A	0.383	0.374	0.431	0.383	0.375*
DRD3	rs2134655	115340891	0.005	1.33	0.007	1.31	A	0.277	0.280	0.226	0.275	0.238*
DRD3	rs963468	115345577	0.008	0.78	0.030	0.82	A	0.381	0.370	0.429	0.375	0.372*
MAOB	rs2283729	43562986	0.042	1.34	0.026	1.26	A	0.274	0.286	0.231	0.289	

*N = nucleotide of minor allele in these samples. *Allele frequencies from unscreened neonatal controls, 1020 chromosomes (Talkowski et al., 2008). **CEU_GENO_PANEL (120 Caucasian chromosomes)*

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